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BODY BUILD AND OXYGEN METABOLISM AT REST AND DURING EXERCISE

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The study of the interrelation of morphological variation with physiological variation has been singularly neglected in the work of the physiologist. With few exceptions, no cognizance has been taken of differences in individual body build in the analysis of human physiological functions. For the most part, the problem of morphological variation has been a matter of simply dealing with the gross body size factor.

If one considers the purely theoretical side, there appear to be no *a priori* reasons for the tacit assumption that the physiological functions of individuals of disparate body builds are the same. For the physical anthropologist, this assumption is at variance with his conception of the highly integrated character of the human organism. Furthermore, the results of this investigation dealing with the problem of body build and oxygen consumption show quite clearly, at least in regard to one physiological function, that this assumption is unwarranted, and that the subject of the interrelation of morphological variation with physiological variation must be given careful consideration.

THE MATERIAL. The data in this report are derived from the studies in the Fatigue Laboratory of Harvard University, where attention is being paid to the physiology of normal man at rest and at work. The number of individuals comprising this series of data is rather small, consisting of 34 adult males whose ages range from 20 to 38. More than one-half of the individuals in the series were students in the Harvard Business School, a few were undergraduate students, and the remainder were members of the Laboratory staff or of the college faculty. Apart from this selection no other considerations with respect to physical condition, athletic ability,

TABLE 1

Anthropometric characters of 34 individuals in the Fatigue Laboratory Series

MEASUREMENTS AND INDICES	RANGE	MEAN P.E.	S.D. P.E.
Measurements			
Age	20 – 38	26.82 ±0.61	5.25 ±0.43
Stature	162.7 –189.7	175.68 ±0.80	6.90 ±0.56
Weight (kilos)	54.7 – 93.4	72.31 ±1.08	9.35 ±0.76
Surface area (Du Bois)	1.63– 2.10	1.87 ±0.02	0.12 ±0.01
Chest circumference (rest)	81.4 –100.5	91.20 ±0.55	4.75 ±0.39
Chest circumference (insp.)	86.1 –105.7	95.79 ±0.55	4.78 ±0.39
Umbilical circumference	64.4 – 88.5	76.80 ±0.70	6.01 ±0.49
Chest breadth	26.2 – 31.4	28.84 ±0.16	1.40 ±0.11
Chest depth	16.7 – 23.3	20.47 ±0.18	1.58 ±0.13
Chest length	30.7 – 38.5	34.80 ±0.24	2.08 ±0.17
Biacromial diameter	36.3 – 45.0	40.15 ±0.22	1.86 ±0.15
Bi-iliac diameter	25.5 – 33.5	28.36 ±0.20	1.75 ±0.14
Lower extremity length	77.1 – 93.3	83.98 ±0.51	4.40 ±0.36
Bizygomatic diameter	133 –151	140.79 ±0.44	3.84 ±0.31
Indices			
Weight/stature	61.2 –110.7	89.70 ±1.34	11.61 ±0.95
Stature/weight	93.0 –132.7	110.62 ±1.26	10.86 ±0.89
Stature/$\sqrt[3]{\text{weight}}$	11.9 – 14.1	12.80 ±0.06	0.54 ±0.04
Span/stature	100.1 –110.4	104.10 ±0.28	2.41 ±0.18
Sitting height/stature	50.4 – 54.1	52.21 ±0.12	1.00 ±0.08
Trunk height/stature	32.0 – 36.7	33.92 ±0.13	1.10 ±0.09
Lower extremity/stature	45.9 – 49.6	47.78 ±0.12	1.04 ±0.09
Tibia/stature	21.6 – 24.9	22.75 ±0.08	0.69 ±0.06
Tibia/lower extremity	43.5 – 51.5	47.57 ±0.15	1.32 ±0.11
Biacromial/stature	21.2 – 24.9	22.86 ±0.27	2.40 ±0.20
Bi-iliac/stature	14.5 – 18.6	16.16 ±0.10	0.89 ±0.07
Biacromial/trunk height	60.0 – 73.8	67.46 ±0.38	3.27 ±0.27
Bi-iliac/trunk height	39.7 – 56.0	47.44 ±0.36	3.11 ±0.25
Chest circumference/stature	46.4 – 57.9	51.96 ±0.32	2.78 ±0.23
Chest length/sitting height	52.3 – 64.5	58.41 ±0.31	2.69 ±0.22
Chest breadth/trunk height	41.2 – 52.1	48.47 ±0.30	2.59 ±0.21
Bi-iliac/biacromial	62.8 – 77.7	70.66 ±0.38	3.28 ±0.27
Thoracic/abdominal	31.1 – 54.3	43.62 ±0.58	5.01 ±0.41
Chest depth/chest breadth	59.1 – 81.7	71.05 ±0.65	5.62 ±0.46
Cephalic index	71.8 – 83.5	77.25 ±0.33	2.82 ±0.23
Facial index	80.4 –100.8	90.29 ±0.81	6.97 ±0.57
Nasal index	46.8 – 76.9	63.24 ±0.72	6.23 ±0.51

social or economic status, were made. They are all supposedly "normal" in nutritional status, physical condition, and physiological function.

Table 1 presents the statistical constants for the pertinent anthropo-

metric characters of the series. It may be seen that these individuals are on the average fairly tall with a mean stature of 175.68 cm., and of moderate weight amounting to approximately 159 pounds (72.31 kilos).

In absolute diameters they may be said to be rather broad in the shoulders, moderate to narrow in the hips, broad-chested, and rather deep in the antero-posterior chest dimension. The height-weight indices indicate rather tall and moderately slender individuals. Virtually all the men were quite lean and did not possess any extensive fat deposits. Other indicial proportions are suggestive of men with proportionately long arms relative to their total body height, with relatively short leg lengths when contrasted with the statures, with distinctly broad shoulders in proportion to the width of the hips, and with chest proportions veering towards the flat side. Their mean cranial proportions are dolichocephalic bordering on mesocephaly, while the facial and nasal indices indicate relatively long and narrow faces, and distinctly long and narrow noses. Without going into any detail, it may be said that the predominant racial element in this group is undoubtedly Nordic, with Mediterranean and Alpine strains following in order of frequency.

Methods. Before dealing with the essential findings of this study a few remarks relative to methodology, somatological and statistical, may well be pertinent. For purposes of morphological description only strict anthropometric characters were utilized, both measurements and indices. At this stage of the investigation the attempt was made to approximate the skeletal proportions or bony framework of the body. No arbitrary somascopic or scopometric classifications are presented. For purposes of measuring the presence as well as the degree of association between oxygen consumption and the various anthropometric measurements, product-moment coefficients of correlation have been computed by the ungrouped method. The interpretation therefore of any of these results must take into account the limitations of this statistical device. Any very low or statistically insignificant coefficient must not be considered as proving the absence of association between the two variable. At best such low figures should be interpreted in the light of having *failed* to demonstrate the presence of any such relationship. At all times there is no implication of causality.

An entirely different statistical technique was used in dealing with the anthropometric indices. Each index range was split into two parts at the mean of the entire distribution, and for each of these divisions the mean oxygen consumptions were computed. Statistical significance of the differences was estimated by Fisher's (1936) analysis of variance method. The values of "F" were taken from table XXXV of the Snedecor publication (1934). These "F" values correspond to Fisher's 5 per cent point, and hence any value exceeding this level is considered to be *significant.*

The resting state. We shall first deal with body build and oxygen consumption in the basal state, that is, after the fasting subject has rested in the reclining position for one-half hour. A very large number of studies have been made with respect to basal metabolism in man. In this regard the work of Harris and Benedict (1919), of Du Bois (1927), and of Boothby, Berkson, and Dunn (1936), are perhaps the best known. It is from these studies that we derive standards for basal metabolic rates, based on surface area or height and weight formulae. These investigations have paid attention to measurements of height, weight, and surface area, but practically no data are available in respect to the interrelation of oxygen consumption with any extensive series of measurements and proportions of the body.

TABLE 2

Coefficients of correlation between anthropometric measurements and oxygen intake

MEASUREMENTS	O_2, CC. PER MINUTE BASAL OR RESTING STATE r ± P.E	O_2, LITERS PER MINUTE	
		Moderate exercise	Severe exercise
		r ± p.e.	r ± p.e.
Age	−0.175 ±0.112	−0.004 ±0.116	−0.190 ±0.111
Stature	0.447 ±0.093	0.363 ±0.100	0.590 ±0.075
Weight	0.412 ±0.096	0.771 ±0.047	0.882 ±0.026
Surface area (Du Bois)	0.505 ±0.086	0.724 ±0.055	0.591 ±0.075
Chest circumference (rest)	0.377 ±0.099	0.817 ±0.038	0.810 ±0.040
Chest circumference (insp.)	0.412 ±0.096	0.787 ±0.044	0.876 ±0.027
Umbilical circumference	0.225 ±0.110	0.687 ±0.061	0.692 ±0.060
Chest breadth	0.362 ±0.101	0.568 ±0.078	0.405 ±0.097
Chest depth	0.424 ±0.095	0.591 ±0.075	0.720 ±0.056
Chest length	0.361 ±0.101	0.394 ±0.098	0.455 ±0.092
Biacromial diameter	0.357 ±0.101	0.377 ±0.099	0.746 ±0.047
Bi-iliac diameter	0.309 ±0.105	0.592 ±0.075	0.870 ±0.027
Lower extremity length	0.303 ±0.105	0.275 ±0.107	0.472 ±0.090
Bizygomatic diameter	0.097 ±0.115	0.542 ±0.082	0.170 ±0.111

In our subjects the resting oxygen intake was determined on the Roth-Benedict apparatus, in which the subjects breathed a gas mixture with oxygen in excess of 7 minutes.

Table 2 gives the coefficients of correlation between oxygen consumption in cubic centimeters per minute and a number of gross body measurements. It may be seen that with the exception of age, all the body measurements presented show positive and fairly high coefficients of correlation. Even though we have attempted to reduce the age factor in oxygen consumption by limiting our series to adult men in the twenties and thirties, we are still left with a low negative association. In other words, with an increase in age there is still a slight decrease in oxygen consumption in this group. Similar results have already been reported for a more extensive distribution

by Robinson (1938). The rest of the figures speak for themselves. Inasmuch as basal oxygen consumption is very highly dependent on size of body mass and surface area, the larger the gross measurements the higher the oxygen consumption. Thus, it may be seen that in our figures, surface area presents the highest coefficient of correlation, followed by stature, weight, and the measurements of the chest.

TABLE 3

Comparison between average oxygen intakes for low index group and high index group

The mean value of the index (see table 1) was taken as the dividing point to separate the entire group into low and high index groups. Oxygen intakes in basal state

INDICES	MEAN O_2 (CC./HOUR/KILO BODY WEIGHT)			MEAN O_2 (LITERS/HOUR/SQ.M BODY SURFACE)		
	Low indices	High indices	Mean difference	Low indices	High indices	Mean difference
Weight/stature	22.97	19.23	3.74*	8.32	7.89	0.43*
Stature/weight	19.28	22.92	−3.64*	7.88	8.33	−0.45*
Stature/$\sqrt[3]{\text{weight}}$	19.96	22.99	−3.03*	8.00	8.29	−0.29
Span/stature	22.09	20.22	1.87*	8.31	7.90	0.41*
Sitting height/stature	20.58	21.77	−1.19	7.91	8.31	−0.41*
Trunk height/stature	20.92	21.58	−0.66	8.11	8.13	−0.02
Lower extremity/stature	21.52	20.86	0.66	8.31	7.91	0.41*
Tibia/stature	21.26	21.14	0.12	8.14	8.08	0.06
Tibia/lower extremity	20.98	21.44	−0.46	8.00	8.24	−0.24
Biacromial/stature	21.62	20.74	0.88	8.13	8.11	0.02
Bi-iliac/stature	22.72	20.02	2.70*	8.28	7.99	0.29
Biacromial/trunk height	21.86	20.63	1.23	8.23	8.02	0.21
Bi-iliac/trunk height	22.14	20.03	2.11*	8.18	8.04	0.14
Chest circumference/stature	22.59	19.82	2.77*	8.25	7.98	0.27
Chest length/sitting height	21.46	20.99	0.47	8.08	8.16	−0.08
Chest breadth/trunk height	21.84	20.65	1.19	8.21	8.03	0.18
Bi-iliac/biacromial	21.95	20.16	1.79*	8.20	8.01	0.19
Thoracic/abdominal	21.52	20.82	0.70	8.22	7.99	0.23
Chest depth/chest breadth	21.81	20.53	1.28	8.16	8.07	0.09
Cephalic index	21.57	20.86	0.73*	8.26	7.96	0.30
Facial index	20.51	21.70	−1.19	8.07	8.15	−0.08
Nasal index	21.95	20.62	1.33	8.13	8.11	0.02

* Statistically significant differences by the analysis of variance method.

On turning to the examination of the various metric proportions it was found necessary to correct the gross oxygen consumption of our individuals for body mass. This was done in order to eliminate the possibility that variations in certain indices went along with well-defined body mass changes. Accordingly, a correction for oxygen consumption was made by relating the oxygen consumption of each individual to his body weight;

computed by dividing the oxygen intake in cubic centimeters per hour, by body weight in kilograms. These figures were then associated with the body build proportions by the adoption of the simple technique of computing the average oxygen intake per kilo of body weight for each half of the frequency distributions of the various indices.* Thus for every body proportion the average oxygen intake per kilo of body weight of all individuals below the mean were compared with similar oxygen intake average for all individuals with indices above the mean. The results (table 3) are interesting and significant, and they may be summarized as follows: Individuals who have more linear body builds have higher oxygen consumptions per kilo of body weight than those persons with more lateral body proportions.[1] Higher oxygen consumptions are shown by those individuals with narrower shoulders when related to stature or trunk height, with narrower hips when related to stature or trunk height, with smaller chest circumferences in proportion to the total body height, with narrower chest breadths relative to the torso height, in individuals with narrower hips relative to shoulder breadth, in persons with chest outlines which are flatter, in those individuals with shorter thoracic cavities relative to length of abdominal cavities, in persons with more dolichocephalic head proportions, and with more leptoprosopic and leptorrhine facial and nasal proportions.

In general then it may be said that those individuals with more linear body builds rather than lateral and those with relatively shorter extremities rather than longer have higher oxygen consumptions per unit of body weight and hence higher basal metabolic rates. Lateral body build individuals appear to be more economical in their consumption of oxygen at rest than the linear type of person. These findings are supported by the work of Lucas and Pryor (1933) who found that children with slender body builds have higher basal metabolic rates than those children possessing more lateral body proportions.

The remarkable consistency of the direction of these differences for the various indicial proportions leaves little doubt as to the general reliability of the results. However, for the sake of completeness, the statistical significance of each of the indicial comparisons has been estimated by Fisher's analysis of variance method. The question to be answered is as follows: is the variation between the means of the index classes to be considered significant, or may these index class differences be ascribed to sampling? For the sake of economy of space these figures are not given in this paper. However the method by which they were obtained is as follows: The mean square values *between the means of the index classes* are compared with those *within the index classes* to give "F"

[1] Hereafter the term "linear" refers to a body build where the lateral dimensions are relatively smaller and narrower rather than larger and broader.

values. These actual "F" values are then contrasted with the expected "F" values for the 5 per cent level. The latter values are expected to be exceeded in random sampling from a homogeneous population only five times in a hundred trials. Accordingly, those indicial proportions showing actual "F" values greater than the 5 per cent expected level may be considered as being statistically significant beyond the error of random sampling.

These "analysis of variance" figures show, in spite of the small size of the series used in this study, that statistically significant differences are to be found between the means of the low and high index classes for all three of the weight-stature proportions, for the bi-iliac/stature index, for the bi-iliac/trunk height index, for the chest circumference/stature, bi-iliac/biacromial, span/stature, and cephalic indices. Differences which approach the 5 per cent level of significance occur in the case of the sitting height/stature, biacromial/trunk height, chest breadth/trunk height, chest depth/chest breadth, and the nasal indices.

The oxygen consumption in the resting state is considered by some physiologists to depend more closely on body surface than on gross body weight. The correctness of this view insofar as different mammals varying greatly in size are concerned is clear. In order to test the applicability of this view to our group, oxygen consumption has been related to body surface area for each individual. Table 3 gives the comparison between the average oxygen intakes in liters per hour per square meter body surface for the *low* indices and *high* indices. These figures show that the trends of the differences between the *high* and *low* indices are virtually identical with those found for oxygen consumption when related to body weight. Again the more linear individuals, and those with shorter extremities, flatter chests, and with longer and narrower heads, show higher oxygen intakes than their morphological opposites. The only disparity appears in the case of the chest length/sitting height index, where the body surface figures give a higher mean oxygen intake for those individuals with greater chest lengths relative to the sitting height in contrast to the body weight computations which present higher oxygen intakes for the relatively shorter chested individuals.[2]

The determination of the statistical significance of the body surface

[2] For those readers who may contend that these body build (indicial) associations with oxygen consumption are simply specious manifestations of the well demonstrated relationship between metabolism and stature, the writer has computed the mean oxygen consumptions per square meter surface area for high and low values of the straight measurements. The results confirm without exception the indicial comparisons given above. Higher mean oxygen consumption values per surface area are found in those individuals with narrower shoulders, narrower hips, narrower chests, shallower chests, smaller chest circumferences, smaller umbilical circumferences, shorter lower extremities, longer chests, and narrower faces.

figures by the analysis of variance method shows that statistical significance is attained by the weight/stature index, by the stature/weight index, by the span/stature, sitting height/stature, and lower extremity/stature indices. Differences which approach the 5 per cent level of significance are obtained for the stature/$\sqrt[3]{\text{wt.}}$ index, the bi-iliac/stature, and cephalic indices.

Moderate exercise. We shall deal next with the association of body build and oxygen consumption during moderate exercise. The following grade of work was performed. Each individual walked on a treadmill for 15 minutes at the rate of 5.6 kilometers per hour on a grade of 8.6 per cent. The volume of air expired was measured in the gasometer, from the end of the 8th to the end of the 14th minute of walk. Duplicate samples of this expired air were drawn from the gasometer and analyzed for O_2 and CO_2. In this manner the oxygen intake in liters per minute was computed for each individual. The average oxygen intake for our series is 1.96 liters per minute ($\sigma = 0.21$) for this grade of work. This value is about 7.8 times the oxygen consumption of our group during the basal or resting state.

Table 2 presents the coefficients of correlation computed between anthropometric measurements and the oxygen intake during the walk. The first thing to be noticed is that in almost all instances these coefficients are considerably higher than those obtained during the basal or resting state. It also appears that age shows no association with amount of oxygen intake during moderate exercise. In the basal state the highest coefficients were obtained for surface area and stature, but here the highest coefficients are those dealing with chest circumference at rest and at maximum inspiration, followed by weight, then surface area, umbilical circumference and the other chest and hip measurements. Stature is surprisingly low down on the list with a coefficient value of 0.363.

The figures dealing with the association of oxygen intake in moderate exercise with gross anatomical proportions are given in table 4 which compares the average oxygen consumption in liters per day per kilo body weight of individuals with low indices with the average oxygen consumption of those subjects with high indices. Thus it may be seen that smaller oxygen intakes are displayed by those individuals who are more lateral than linear, who have longer arms relative to their stature, who possess shorter relative sitting heights and trunk heights, longer legs relative to their total body heights, and longer leg segments in comparison to the upper leg segments or total stature. Smaller oxygen intakes are shown by those persons with relatively broader shoulders and hips, by those individuals with relatively larger chest dimensions, by subjects with broader hips relative to the breadth of the shoulders, with longer thoracic cavities relative to length of abdominal cavities, and with rounder rather than flatter chests. And finally, smaller oxygen consumption seems to be

associated with longer and narrower heads, shorter and broader faces, and shorter and wider noses.

Statistically significant values are obtained by the analysis of variance method for the weight/stature index, and for the tibia/stature index. Differences approaching the 5 per cent level of significance appear in the

TABLE 4

Comparison between average oxygen intakes for low index group and high index group

The mean value of the index (see table 1) was taken as the dividing point to separate the entire group into low and high index groups

INDICES	MODERATE EXERCISE			SEVERE EXERCISE		
	Mean O_2 (liters per day per kilo body weight)					
	Low indices	High indices	Mean difference	Low indices	High indices	Mean difference
Weight/stature	40.46	37.69	2.77*	70.64	63.17	7.47*
Stature/weight	38.08	40.12	−2.04	64.34	69.60	−5.26*
Stature/$\sqrt[3]{\text{weight}}$	38.54	40.04	−1.50	65.20	69.87	−4.67
Span/stature	39.79	38.44	1.35	67.97	66.18	1.79
Sitting height/stature	39.04	39.26	−0.22	66.72	67.48	−0.76
Trunk height/stature	38.69	39.75	−1.06	67.19	67.05	0.14
Lower extremity/stature	39.26	39.04	0.22	67.48	66.72	0.76
Tibia/stature	40.13	37.76	2.37*	67.43	66.69	0.74
Tibia/lower extremity	39.85	38.46	1.39	68.35	65.90	2.45
Biacromial/stature	39.72	38.53	1.19	67.67	66.51	1.16
Bi-iliac/stature	40.01	38.48	1.53	68.18	66.29	1.89
Biacromial/trunk height	39.44	38.91	0.53	67.81	66.52	1.29
Bi-iliac/trunk height	40.06	38.01	2.05	67.11	67.15	−0.04
Chest circumference/stature	39.68	38.64	1.04	69.42	64.84	4.58
Chest length/sitting height	40.01	38.39	1.62	68.58	65.84	2.74
Chest breadth/trunk height	39.66	38.71	0.95	68.82	65.62	3.20
Bi-iliac/biacromial	39.69	38.39	1.30	67.72	66.28	1.44
Thoracic/abdominal	39.72	38.44	1.24	68.27	65.68	2.59
Chest index	39.76	38.48	1.28	67.59	66.60	0.99
Cephalic index	38.82	39.53	−0.71	66.84	67.45	−0.61
Facial index	38.29	39.76	−1.47	64.59	68.90	−4.31
Nasal/index	39.44	38.92	0.52	67.47	66.86	0.61

* Statistically significant difference by the analysis of variance method.

case of the stature/weight, bi-iliac/trunk height, and the chest length/sitting height indices. While the small size of the series militates against demonstration of statistical significance for many other bodily proportions, the consistency of the trends and directions of the differences is impressive.

The fact that there appears to be a relationship between body build and oxygen consumption during moderate exercise, raises the question as to the degree of mechanical efficiency with which this sort of work is done. Do the more "lateral" body build individuals, who consume less oxygen

per kilo of body weight, possess greater mechanical efficiency in grade walking on the treadmill than the more "linear" type? In order to answer this question, mechanical efficiency values were calculated for each individual by the following formula:

$$\text{M.E.} = \frac{\text{Work Output}}{\text{Energy Input}} = \frac{\text{Work done} \times 100}{(\text{Total energy}) - (\text{Basal Energy Exchange})}$$

Work = (Body wt. in kg.) (Vertical lift in m.)
Total energy = Total kg.m. calculated from O_2 intake.
Basal energy = Basal kg.m. calculated from the Basal O_2 intake.

For each of the indicial proportions average mechanical efficiency values were computed for the *low* indices and the *high* indices. Table 5 presents these figures. If we first consider the weight-stature indices we find that greater mechanical efficiency is shown by those individuals who are more lateral in body build than linear. Greater mechanical efficiency is displayed by those individuals with relatively longer arms, with greater lower leg length relative to stature as well as to the total lower extremity length; by those persons with broader shoulders and broader hips relative to their respective statures, with greater chest circumferences and chest lengths relative to their total body heights, and with broader hips in comparison to the shoulder width. And finally, greater mechanical efficiency in grade walking is possessed by those subjects who have relatively greater chest cavities in comparison to size of abdominal cavities, by those individuals with rounder rather than flatter chests, by those persons with longer and narrower heads, broader and shorter faces, and longer and narrower noses.

Statistically significant differences are obtained for these comparisons in the case of the weight/stature index, and for the chest length/sitting height index. Differences approaching the 5 per cent level of significance are suggested in the following proportions: stature/weight index, tibia/stature index, thoracic/abdominal index, and facial index.

It would appear then that not only do those individuals with the more lateral type of body build consume less oxygen per kilo of body weight than the linear type during moderate exercise, but they also possess greater mechanical efficiency in work performance.

Exhausting work. Maximal oxygen intake was obtained on all the subjects during 2 to 5 minutes of exhausting running on the treadmill. The speed of the treadmill was increased so as to exhaust most individuals within the 5 minute period. Just as in the case of the walking exercise, the expired air was collected in a 500 liter gasometer and samples of the air analyzed. The average maximal oxygen intake for this most severe form of work was 3.35 liters per minute, or almost twice the average oxygen intake for the more moderate form of exercise.

Table 2 gives the coefficients of correlation for the maximal oxygen

intake and the various anthropometric measurements. It may be seen that as the age of the group increases there is a slight decrease in the maximum amount of oxygen intake for this severe form of exercise. All the other measurements are positively correlated to a very high degree.

Weight shows the highest coefficient value, followed very closely by chest circumference at inspiration and bi-iliac diameter. Other very high

TABLE 5

Comparison between average mechanical efficiency for low index group and high index group

The mean value of the index (see table 1) was taken as the dividing point to separate the entire group into low and high index groups

INDICES	MEAN MECHANICAL EFFICIENCY†		
	Low indices	High indices	Mean difference
Weight/stature	15.04	16.10	−1.06*
Stature/weight	15.93	15.19	0.74
Stature/$\sqrt[3]{\text{weight}}$	15.62	15.43	0.19
Span/stature	15.42	15.68	−0.26
Sitting height/stature	15.45	15.62	−0.17
Trunk height/stature	15.66	15.39	0.27
Lower extremity/stature	15.62	15.45	0.17
Tibia/stature	15.23	15.99	−0.76
Tibia/lower extremity	15.31	15.78	−0.47
Biacromial/stature	15.44	15.65	−0.21
Bi-iliac/stature	15.41	15.64	−0.23
Biacromial/trunk height	15.57	15.52	0.05
Bi-iliac/trunk height	15.35	15.79	−0.44
Chest circumference/stature	15.32	15.76	−0.44
Chest length/sitting height	14.99	16.03	−1.04*
Chest breadth/trunk height	15.43	15.64	−0.21
Bi-iliac/biacromial	15.50	15.60	−0.10
Thoracic/abdominal	15.18	16.00	−0.82
Chest depth/chest breadth	15.27	15.85	−0.58
Cephalic index	15.83	15.21	0.62
Facial index	15.98	15.24	0.74
Nasal index	15.65	15.45	0.20

* Statistically significant difference by the analysis of variance method.

† For mechanical efficiency (M.E.) derivation see formula in text (p. 10).

correlations are displayed by chest circumference at rest, biacromial diameter and chest depth. Surface area and total body height although they present high values are nevertheless quite far down the list.

Table 4 compares the average maximal oxygen intakes per kilo of body weight for the low indicial values with the averages for the high indicial values. It must be remembered that in this form of exhausting work we

are measuring the maximum capacity of the individual for supplying oxygen to the tissues. Hence, high oxygen intake values indicate very effective mechanisms, respiratory and cardio-vascular, for supplying oxygen to the tissues, while low oxygen intake values indicate less efficient mechanisms.

If we run down the list of anthropometric proportions we discover that those individuals who have a more linear body build than lateral, have greater capacity for supplying oxygen to the tissues in this exhausting work. Greater capacities for supplying oxygen are shown by individuals possessing shorter arms and shorter legs relative to their total body heights, by individuals with greater relative sitting heights but not relative trunk heights, by persons with shorter lower leg segments relative to upper leg segments, by men with narrower shoulders and hips relative to their statures. Greater capacities for supplying oxygen are displayed by individuals with smaller chest measurements relative to their statures, sitting heights, or trunk heights; by persons with flatter rather than rounder chests; by individuals with shorter thoracic cavities relative to length of abdominal cavities. And finally, greater capacities for supplying oxygen are possessed by those subjects with rounder heads, shorter and broader faces, and longer and narrower noses.

According to the analysis of variance figures the only statistically significant differences between high and low means occur in the case of the weight/stature, and stature/weight indices. Differences which approach the 5 per cent level of significance are manifested for the stature/$\sqrt[3]{\text{wt.}}$, chest circumference/stature, and facial indices. A most unusual circumstance occurs in the case of the trunk height/stature and bi-iliac/trunk height indices where the variation within the classes is significantly greater than the variation between the means of the low indices and the high indices. That this is an important fact there is no doubt whatsoever, but how to interpret it is a difficult matter and which perforce must be postponed until further study of this unusual phenomenon. It may be possible that some strong bond exists between maximal oxygen intake during severe exercise and trunk height/stature and bi-iliac/trunk height indices.

SUMMARY AND CONCLUSIONS

We may summarize the results of this investigation with respect to the interrelation of body build and oxygen metabolism as follows:

1. There appears to be a relation of a constitutional nature between morphology of individuals and their consumption of oxygen.

2. The more "linear" individuals and those with relatively shorter upper and lower extremities, longer torsos, flatter chest outlines, and with narrower hips relative to breadth of shoulders have higher oxygen intakes in the resting state than their "lateral" counterparts. This appears to be

the case irrespective of whether the oxygen consumption is related to body weight or surface area.

3. With respect to moderate exercise smaller oxygen intakes per kilo of body weight are displayed by individuals who are more "lateral" than "linear."

4. In moderate exercise, greater mechanical efficiency is shown by those individuals whose body build is more "lateral" than "linear."

5. In exhausting work on the treadmill, greater capacity per kilo of body weight for supplying oxygen to the tissues is shown by those individuals who are more "linear" than "lateral," and who possess shorter upper and lower extremities and longer torsos.

6. And finally, there seems to be a racial variation as expressed by the cranio-facial indices with regard to oxygen metabolism, the exact ramifications of which are not yet clear.

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THE CARDIAC VAGUS NERVE OF THE FROG AS AFFECTED BY SODIUM N-HEXYLETHYL BARBITURIC ACID (ORTAL)[1]

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A discussion of the literature on the action of the barbiturates on the cardiac vagus nerve in mammals has been previously presented by us (1) and is, therefore, omitted here. That all barbituric acid derivatives depress the cardiac vagus nerve in the terrapin was shown by Gruber, Haury and Gruber (2). The point of action of these drugs in paralyzing this nerve was also studied by these authors (3) and they showed that sodium ortal, sodium evipal and sodium amytal all paralyze the post-ganglionic nerve fibers. This was revealed by electrically stimulating pre- and post-ganglionic fibers of the vagus before and after the application of the barbiturate to the heart and comparing the results thus obtained with those secured with nicotine. Since pre- and post-ganglionic nerve fibers of the vagus nerve can be stimulated in the frog, it was thought of interest to see if the results obtained on the terrapin could be supported by experiment on the frog.

METHOD. Frogs of the species Rana pipiens were used in this research. The animal, pithed and with the spinal cord destroyed, was fastened to a frog board with the ventral surface up. The right vagus nerve was exposed and prepared for electrical stimulation. The heart was also exposed and the tip of the ventricle was fastened to a light muscle lever. For stimulation of the post-ganglionic vagus nerve fibers to the heart, platinum stimulating electrodes were fixed to the white crescent. Two inductoria were used, one for stimulation of the vagus and another for excitation of the crescent. In both instances the strengths of the currents were such as to produce either marked slowing or complete arrest of the heart. The strength of the current was not altered until the completion of the experiment. Ortal sodium, dissolved in Ringer's solution, making M/1000 and M/500 dilutions, each having a pH of 7.8, was dropped on the ventricle, auricles and sinus for a period of 2 to 4 minutes. After this all excess fluid was removed from the heart and both the vagus nerve

[1] This research was made possible through a grant by Parke, Davis and Company for research in Science.

and the crescent were stimulated separately as in the control experiments. After this the heart was washed with fresh Ringer's solution (the Ringer's solution was allowed to flow in drops over the heart) until the nerve fibers again responded to electrical excitation as in the control experiments.

RESULTS. Out of twenty-five animals used only 11 experiments were successfully completed because of the delicacy of the vagus nerve and of the white crescent and the fact that in many cases the heart muscle lost

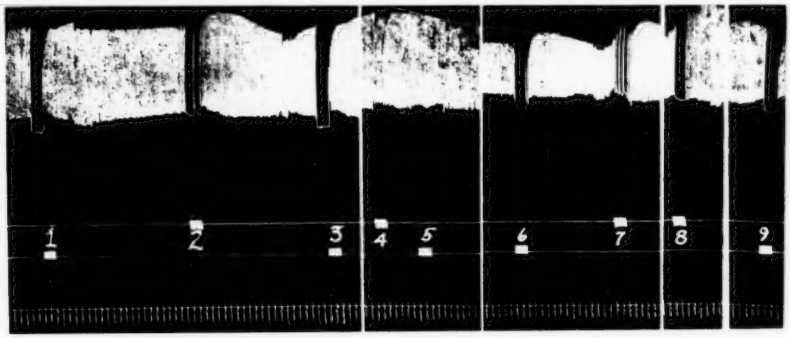

Fig. 1. Top record is that of the frog's heart, below it the time and duration of stimulation of the vagus nerve trunk. The bottom line the time in intervals of 10 seconds and above it the time and duration of excitation of the white crescent. Although the irritability of the vagus and crescent were tested at approximately 5-minute intervals, for the sake of publication, sections of the record are omitted between 3 and 4, 5 and 6, and 7 and 8. In the heart record the upstroke is systolic contraction.

1. Crescent stimulated.

2. Vagus nerve stimulated. The single contraction of the heart seen in this record was due to a temporary stoppage of the stimulation.

3. Stimulation of the crescent. 4 and 5, stimulation of the vagus nerve trunk and crescent respectively after applying sodium ortal to the heart. 6 and 7, stimulation of the sinus and vagus respectively after washing the heart with Ringer's solution for more than 35 minutes. 8 and 9, show the effects of stimulation of the vagus nerve and crescent respectively after washing of the heart with Ringer's solution for 48 minutes.

its force of contraction and ceased beating entirely after about an hour's experimentation. Figure 1 is presented as typical of all the completed experiments. In this figure at 1 and 3 the white crescent and at 2 the vagus nerve trunk were stimulated before the application of the drug to the heart as controls. Ortal sodium M/1000 was applied to the heart between 3 and 4 and at 4 the vagus nerve was stimulated 2 minutes after the drug had been placed on the heart and at 5 the white crescent was stimulated 3 minutes after the application of the substance. In both

instances complete block is noted. The heart was then washed with fresh Ringer's solution and tests made of the vagus and crescent every 5 minutes. After washing for 38 minutes the crescent was stimulated at 6 and the vagus trunk 2 minutes later at 7. Marked recovery is noted in each. At 8 the vagus was stimulated 48 minutes after the ortal was removed and the crescent was excited at 9, 5 minutes later. Complete recovery is noted in each. It appears from these results that ortal sodium depresses the post-ganglionic fibers of the cardiac vagus nerve and that its action is not restricted to the ganglion between the pre- and post-ganglionic fibers.

SUMMARY

1. Sodium N-hexylethyl barbituric acid in dilute solutions when applied to the frog's heart paralyzes the post-ganglionic vagus nerve fibers.

2. These results on frogs support the finding of Gruber, Haury and Gruber on the terrapin heart that though the action may be on the ganglion, it is not confined to this structure but also involves the post-ganglionic nerve fibers of the cardiac vagus nerves.

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THE DURATION OF INSULIN ACTION[1]

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In a previous paper (1) from this laboratory it was shown that the action of insulin on the body which removes glucose from active circulation can be accurately quantitated in the depancreatized dog. After a given dose of insulin the activity can be balanced by intravenous glucose and the rate at which this must be given is a measure of the insulin activity at that particular time. It was shown that the activity became maximal immediately after the intravenous injection of the insulin and thereafter diminished in a steady fashion. The view was also presented that the successive decreases in activity correspond to decreases in the amount of insulin remaining in the body. The present paper is concerned with the determination of the rate at which insulin is removed from the body after injection. The problem is not simple since there is no direct method of assaying the insulin content of the body without sacrificing the animal. The only indirect method is that of determining the insulin activity at the point of time in question. However, the relationship between the amount of insulin in the body at any given instant and the insulin activity resulting therefrom is not a direct proportion (1). In order to assay the insulin of the body by this indirect method it would be necessary to construct an "amount-activity" curve for each experimental animal. This could only be done by the laborious method of giving a great number of insulin doses of different amounts and determining the resultant activity immediately after injection.

For this reason this indirect method of assay of insulin in the body was not used for determining the rate of its disappearance. Another method was adopted. This consisted of determining the "duration of action" of a large number of doses of insulin given to diabetic dogs. This "duration of action" may be readily understood by referring to the previous paper from this laboratory (1). Beginning immediately after a dose of insulin one must inject glucose intravenously in order to maintain the blood sugar constant. The amounts of glucose needed for this steadily

[1] The insulin used in this work was contributed by the Eli Lilly Company.

decrease as time goes on. Finally the blood sugar level is steady without the need of any intravenous glucose but this state of affairs lasts only an hour or so and then the blood sugar begins to rise although no sugar is injected. When this occurs we say the action of the given dose of insulin is over. Strictly speaking one must say that a small amount of insulin remains in the body but it must be less than that which is present when the animal is receiving constant insulin at the "basal rate" (2) which itself is relatively small. In other words the end of the "duration" of action occurs when the amount of insulin becomes less than a definite but very small value. Definite information about the rate of removal of insulin from the body can be obtained from the mathematical consideration of the durations of action of doses of varying amounts. This will be taken up after the results are given.

METHODS. We used depancreatized dogs entirely in our work in order to eliminate the unknown amount of insulin secreted by the pancreas. After pancreatectomy the animals were carried on insulin for one month to allow for complete recovery. Thereafter they were maintained at a constant weight on insulin and a measured diet of raw pancreas, meat and sugar with adequate vitamins and a small amount of sodium chloride. A given dog was not used more than once a week for a determination.

For the test the dog received his last food and insulin 24 to 36 hours before the start of the run. Some insulin was given intravenously the previous evening. On the morning of the experiment two or more units of insulin were given intravenously. The purpose of this was to bring the blood sugar level down to normal; the amount needed was established by previous trial, and once determined was quite constant for a given animal. Blood sugar determinations were made every 30 minutes by the Folin-Wu method. After the blood sugar had reached a normal or subnormal level and was rising again, the test proper was begun. For the tests insulin was always given intravenously.

From then on glucose was given intravenously at a rate just adequate to maintain the blood sugar at a constant normal value. Frequent blood sugar determinations were carried out to control this and proper adjustments in the rate of injections were made as indicated by the changes in blood sugars. As stated above the glucose necessary became steadily less as time went on until a short period supervened during which no glucose was needed. After this the blood sugar began to rise and this was taken as the end of the action of the dose.

Thirty measurements were carried out on 7 depancreatized dogs with doses ranging from 2 to 100 units. It was evident to us that although the duration of action of larger doses was longer than smaller, it was in no ways proportionally so. Thus the duration of action of 100 units is not 10 times that of 10 units but rather somewhat less than double. For this reason the

results are given with the duration plotted against the log of the dose in figure 1. Represented in this way the results group themselves along a straight line AB. This means that the rate of removal of the insulin is greater when larger amounts are present in the body. That the duration of action should be closely proportional to the log of the dose suggests that the rate of removal at any instant is directly proportional to the amount of insulin in the body at that time.

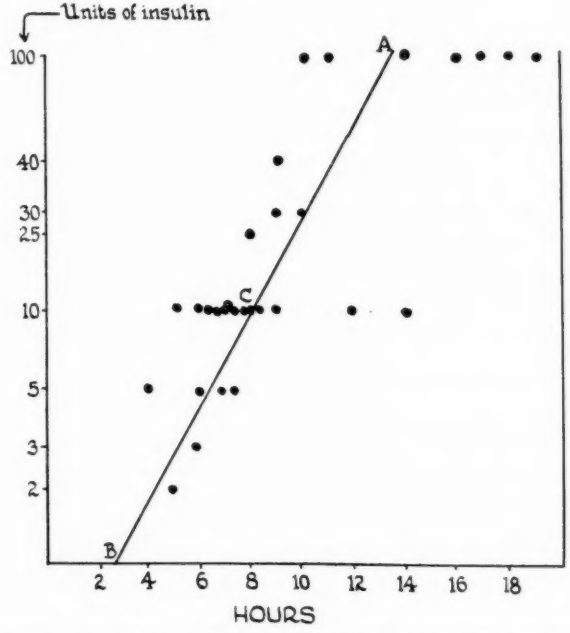

Fig. 1 shows the relationship between the dosage of insulin (the distance up on the ordinate is proportional to the logarithm of the dose) and the duration of action of the dose.

The mathematical treatment of these results leads to some interesting and possibly useful values. Naturally with a scatter of points of this nature no high precision in figures can be obtained, but first approximations may certainly be arrived at which are of use. Such a relationship as that suggested above—namely, that the rate of removal at any instant is directly proportional to the amount of it in the body at that time, is expressed mathematically by the equation: $\frac{da}{dt} = -ka$, in which a is the amount of insulin in the body at any instant, k is a constant and $\frac{da}{dt}$ represents the rate

at which insulin is disappearing from the body at the instant a units remain in it. On integrating the above equation we obtain $\log a = Ce^{-kt}$ in which C is the value of a when $t = 0$, in other words the amount of insulin injected. t in the above equation may be expressed in any unit; it is most convenient to express it in hours which we shall do hereafter, hence k would also be expressed on a per hour basis.

When, after a given dose, the insulin in the body (a in the equation) diminishes to less than that present in the body when the animal is receiving it at a basal rate, the blood sugar will rise, and at this point we say the "duration of action" is over. The line AB in figure 1 gives the average durations of doses of various amounts. During the times indicated by the line the doses of insulin diminish to less than that that must be in the body for the basal requirement. Thus the time indicated by the point A (13.8 hrs.) is that necessary for the dose of 100 units to decrease just to less than that present in the animal when it is receiving basal insulin (designated hereafter as A_b). Since this latter amount is the same for all points along the line AB, we may supply it in the general equation $a = Ce^{-kt}$ for any 2 points on the line and so solve for the unknown k. (At all points on the line, A_b equals Ce^{-kt} and C and t are known. This gives us a value for k of 0.4 per hour and since k is the constant of the original equation, $\frac{da}{dt} = -ka$, this means that at any instant insulin is disappearing from the body at the rate of four-tenths of the amount present per hour. We may then solve for A_b which comes out equal to 0.4 unit. During the time indicated by any point along the line AB the insulin of the given dose would have decreased to this value. As stated above this is slightly less than the amount of insulin in the body of the average diabetic dog when it is receiving it at the basal rate.

The average basal rate for 12 diabetic dogs was found by Greeley (2) to be 0.189 unit per hour. If we take the value for k above as 0.4 per hour we would find that a dog that had received insulin at the rate of 0.189 unit per hour for 6 hours (the interval usually used for basal rate determinations) would have an amount of insulin in his body averaging 0.43 unit. This agrees well with the value estimated by the method used above, i.e., 0.4 unit. The close agreement is probably somewhat fortuitous since, as mentioned above, there is considerable scatter of points in figure 1 and the figure for average basal rate is obtained from values which vary considerably. However, the fact that the two values, obtained by quite different procedures, should be of the same order of magnitude is significant and gives definite support to the assumptions which we have made regarding the mechanism of the action of insulin.

The assumptions may be recapitulated here:

1. The action of insulin on carbohydrate metabolism is twofold. The

"basal" insulin prevents excessive new formation of glucose. Doses above the "basal" cause removal of sugar from the blood; this activity can be quantitated by determining the rate at which glucose must be injected intravenously to balance this removal.

2. The intensity of the activity at any instant after a given dose of insulin is dependent on the amount of insulin remaining in the body at that time.

3. Insulin is removed from or destroyed by the body at any instant at a rate proportional to the amount present at that time.

Discussion. The rate of decay of insulin occupies a position intermediate between such rapidly destroyed hormones as adrenin and vagus-substance on the one hand, and thyroxin, with a relatively slow rate, on the other. Boothby et al. (3) have made a quantitative study of the decay curve of thyroxin, using its calorigenic effect on a myxedematous subject as a measure of its activity. They obtained the equation, $H = H_0 \times 10^{-0.024t}$ which indicates the decay curve of thyroxin. In this equation H = excess heat calories due to thyroxin, H_0 is the heat produced for zero day (for the particular patient and dose they used equal to 1660 calories per day) and t is the number of days following the zero day. The decay curve for insulin is similar to this but much more rapid.

SUMMARY

For the depancreatized dog the duration of action of an intravenous dose of insulin depends on the size of the dose.

For doses of physiological magnitude the duration of the action is proportional to the logarithm of the size of the dose.

The rate at which insulin is destroyed in the body at any instant is proportional to the amount of it in the body at the time. The average rate is four-tenths of this amount per hour or one nine thousandth of the amount per second.

Acknowledgment is made to Dr. D. R. Drury for help in the mathematical treatment of the data.

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THE ELECTRICAL EXCITABILITY OF MAMMALIAN STRIATED MUSCLE

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Prolonged indirect stimulation of skeletal muscles in cats and dogs results in a late persistent rise of tension (5th stage of neuromuscular transmission, Rosenblueth and Luco, 1939) which follows the lack of transmission during the period of fatigue (4th stage). In a previous communication (Rosenblueth, Lissák and Lanari, 1939) it was shown that the chemical theory of neuromuscular transmission readily explains both the 4th stage, as due to a decrease of the acetylcholine output from the motor nerves, and the 5th stage, corresponding to a late increase of this output.

The electrical theory of transmission suggests only two possibilities for the recovery which occurs in the transition from the 4th to the 5th stage: either the nerve action potentials or the electrical excitability of the muscle should increase. Rosenblueth and Luco (*loc. cit.*) studied the magnitude of the nerve spike potentials over prolonged periods of stimulation at various frequencies and found either no significant change or a slight decrease, but never an increase. The present study had as its original purpose the determination of the muscular electrical excitability during the various stages of neuromuscular transmission.

The methods employed thus far to distinguish the electrical excitability of normal muscles from that of their motor nerves (Rushton, 1932; Grundfest, 1932) were considered unsatisfactory for the present purpose. A method was devised, therefore, which readily separates the muscular responses to direct from those to indirect activation. Since the majority of the previous studies of muscular excitability have been made on frogs instead of mammals, and since many of the problems involved in those studies have raised serious controversies, it was deemed important to extend the present inquiry to include some of those controverted questions.

METHODS. The majority of the experiments were made on cats under dial anesthesia (Ciba, 0.75 cc. per kgm. intraperitoneally). For purposes of comparison a few observations were made on frogs, after pithing.

[1] Fellow of the Rockefeller Foundation.

The electric responses of the muscles were led to suitable direct-coupled or capacity-coupled amplifiers and observed or photographed from a cathode-ray oscillograph. The distinction between direct and indirect stimulation of the muscles was based on the latency of the responses. While the effects of indirect stimulation are not apparent until the lapse of the neuromuscular delay, the responses to direct activation do not have such a delay, but follow the stimulus immediately.

Conduction in muscle being slower than in nerve, if the responses were recorded at some distance from their site of origin the temporal relations might become confused or even inverted, the "indirect" response preceding the "direct" component in the records. To avoid this source of error one of the recording electrodes was usually in the close vicinity of the stimulating cathode. The procedure in these cases was to insert the anode in some distant region of the body, in order to insure a relatively localized origin for the recorded responses. It was found, however, that it was possible as a rule to distinguish clearly the "direct" and the "indirect" components of the electrograms even when both stimulating electrodes were placed outside the recording muscle. The several tests made to control the validity of the interpretation of the electrograms, and therefore the method, are reported below in section A.

Since the mechanical responses elicited were either just liminal or quite submaximal no precautions were usually taken for fixing the recording muscles. Observations of electric responses were made on vastus lateralis, sartorius, gastrocnemius, soleus and some of the foot muscles (lumbricales) after section of the corresponding nerve supply. When mechanograms were desired, either gastrocnemius or soleus was used. The leg was fixed by drills inserted into the tibiae and the freed tendons were attached to tension myographs pulling against heavy rubber bands.

The electrodes used for stimulating the muscles or recording from them were silver or platinum needles. For stimulation of the motor nerves shielded silver-wire electrodes were applied to them distally to the cut which disconnected them from the central nervous system.

The stimuli used for the construction of voltage-capacity curves describing the electrical excitability were condenser discharges of from 10 to 0.0001 μF capacity. Various resistances were usually placed in series and in parallel with the stimulated tissue. Whenever the resistance of the stimulated tissue was of significance in determining the total resistance of the discharging circuit it was determined by measuring on the cathode ray oscillograph the time-constant for discharge of various capacities to $1/e$ of their initial voltage. Repetitive stimulation of the motor nerves was obtained by the diphasic output of a transformer which received condenser discharges at a frequency regulated by a thyratron.

The voltage-capacity curves were constructed for constant submaximal

or for just threshold responses, when this threshold was sharp. The curves were analyzed as suggested by Hill (1936), thus obtaining values both for the rheobase (r) and for the time constant (k), which is proportional to the chronaxie. The fit of the theoretical curve with the experimental curves obtained from direct stimulation was invariably satisfactory in the ranges observed. The curves obtained by indirect stimulation did not fit the theoretical law as accurately as did those derived from direct stimulation (see Rosenblueth and Dempsey, 1939). Even with such an imperfect fit, however, Hill's treatment provides a simple system for uniquely describing the curves by the values of r and k. The results will be reported, therefore, on the basis of those values.

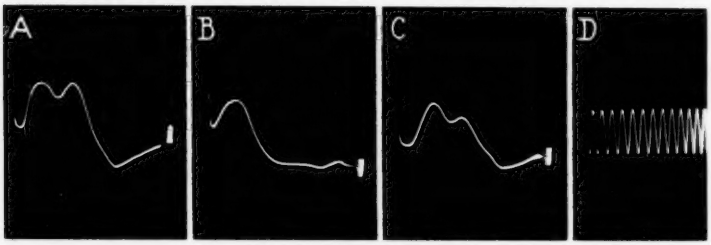

Fig. 1. Persistence of the direct component and abolition of the indirect component of the muscle electrograms by curare. Cat's vastus lateralis of quadriceps. One of the recording electrodes 2 mm. away from the stimulating cathode. In these and the other electrograms the stimuli were made to start the sweep of the electron beam in the cathode-ray oscillograph. The records begin, therefore, with the stimulus artifact.

A. Before curare. Response to a condenser discharge (0.001 μF, 55 v.). Both the direct and the indirect components were present.

B. After curare (0.001 μF, 70 v.). The indirect component disappeared.

C. One hour later (0.001 μF; 60 v.): the animal was largely decurarized. The indirect component was again present.

D. Calibration of the speed of the sweep: 2,000 cycles.

The drugs employed were curare (the crude Brazilian product) and prostigmin (Roche). When the latter substance was used, atropine (sulphate, Merck) was injected at the beginning of the experiment to prevent the parasympathomimetic action. The injections were made intravenously. Artificial respiration was administered through a tracheal cannula, when necessary.

Results. A. *Tests for the validity of the method.* In order to ascertain that a difference in delay would allow the recognition of two components of the electrograms due to direct and indirect stimulation of the muscles, respectively, the following tests were made.

Curare should abolish the response attributed to nerve activation,

while leaving unchanged that due to direct stimulation of the muscle. This was invariably confirmed (fig. 1).

Repetitive stimulation at a sufficiently rapid rate to produce failure of neuromuscular transmission (Wedensky inhibition) should similarly abolish the indirect component of the response and leave the direct action relatively unimpaired. This was confirmed after injections of prostigmin or small doses of curare, insufficient to block transmission. In either of these two cases Wedensky inhibition takes place with slow frequencies of stimulation (see Rosenblueth and Morison, 1937a, for references).

If the repetitive discharges elicited by single nerve impulses after prostigmin are due to persistence of acetylcholine liberated at the nerve endings, as was suggested by Brown, Dale and Feldberg (1936), and not due to a change in the muscle fibers (Eccles, 1936), then stimuli which

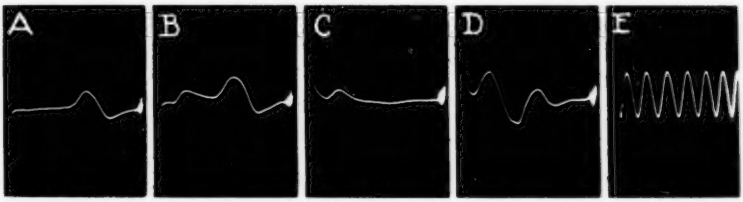

Fig. 2. Differential effects of α and γ stimuli upon the direct and indirect components of the electrograms. Sartorius. Stimulation from a needle in the pelvic end, *a*, to one in the middle of the muscle, *b*. Record from a needle, *c*, 3 mm. below *b* to another, *d*, at the tibial end of the muscle. A and B were obtained with the stimulating cathode at *a*; shocks: 0.02 μF, 3 v. and 0.02 μF, 9 v., respectively. In C and D the cathode was at *b*; shocks as for A and B. E, 1,000 cycles.

evoke only the direct component should not be followed by repetitive activity. This was found to be the case (figs. 8 and 9).

Finally, since the α and γ segments of the voltage-capacity curves of muscles stimulated with diffuse electrodes correspond to direct and indirect activation, respectively (Lucas, 1907; Rushton, 1932a and b), threshold α stimuli should evoke only the direct component and γ stimuli only the indirect component of the electrograms. In figure 2 are shown the differential effects of α and γ stimuli in a cat sartorius. Weak γ shocks in A elicit an indirect response; the direct component appears only upon intensification of the stimulus (B). Conversely, α shocks do not activate the nerve (C) until strengthened (D). Figure 3 shows α and γ curves constructed from the electrograms of a frog sartorius stimulated *in situ*. Diffuse stimulation was obtained by placing the electrodes outside the muscle, the cathode in the abdominal wall and the anode in the leg, below the knee.

B. *The neuromuscular delay.* The neuromuscular delay has been only

little studied in mammals. The methods employed for this study have involved the computation of time for conduction in a significant segment of nerve. The present method afforded an opportunity of rendering practically negligible the conduction time in nerve and muscle, by applying fairly intense stimuli and recording close to the stimulating cathode.

Accurate measurements could be made only for the fastest elements contributing to the direct and indirect components of the electrograms. In table I are listed the values of the briefest neuromuscular delays encountered in sartorius and gastrocnemius in several experiments. That neuromuscular delays may be much longer than those listed here was shown by another method in the facial muscles (Rosenblueth and Morison, 1937b).

TABLE I

Neuromuscular delays (in msec.)

In the columns headed D and I are listed the delays after application of the stimulus for the beginning of the "direct" and "indirect" components of the electrograms, respectively. The neuromuscular delay is the difference between these two values.

	SARTORIUS			GASTROCNEMIUS		
	D	I	Delay	D	I	Delay
	0.97	2.95	1.98	0.65	1.55	0.9
	0.28	1.55	1.27	0.65	1.35	0.7
	0.28	1.73	1.45	0.50	1.50	1.0
	0.46	2.15	1.69	0.75	1.40	0.65
	0.39	2.15	1.76	0.85	1.75	0.9
	0.40	1.70	1.30	0.65	1.50	0.85
	0.50	2.10	1.60	0.40	1.15	0.75
Av.	0.47	2.05	1.58	0.64	1.46	0.82

C. *The electrical excitability of normal muscles.* As is well known, the measurements of the electrical excitability of muscle are influenced by several factors. The magnitude of the stimulating electrodes plays a rôle (Davis, 1923). This factor was significant in the present observations when comparing the results obtained with stigmatic stimulating electrodes in the muscle with the observations in which the electrodes were placed outside the recording muscle, thus insuring diffuse stimulation. The time parameter k was greater in the latter condition. The influence of the type of electrodes used was also noticeable in the measurement of the electrical excitability of the intramuscular nerve fibers: diffuse stimulation yielded greater values of k than did activation with stigmatic electrodes placed in the neighborhood of those fibers.

The relative position of the anode and cathode with respect to the recording muscle fibers was likewise capable of seriously modifying the measured electrical excitability. Thus, when the stimulating needles were placed at the tendon and the belly, and the recording needles were inserted between them, changes in the polarity of the shocks delivered resulted in significant changes of k.

The magnitude of the response selected as an end-point in the measurements—i.e., the number of elements activated by the stimuli—also plays a rôle in the value of k (see Rosenblueth and Rioch, 1933). Less excitable

TABLE 2

Influence of the mode of stimulation on the excitability parameters of nerve and muscle

Observations made on 4 muscles. In the column headed "Indicator" the letters have the following meaning: M, mechanical response; E, electrogram; D, direct component; I, indirect component.

MUSCLE	MODE OF STIMULATION	INDICATOR	r (VOLTS)	k (MSEC.)
Gastrocnemius	Popliteal nerve	M	0.40	0.055
	Muscle; − at belly, + at tendon	M	0.53	0.049
	Muscle; + at belly, − at tendon	M	1.30	0.168
	Muscle; − at belly, + at tendon	ED	1.70	0.092
	Muscle; − at belly, + at tendon	EI	1.20	0.053
Gastrocnemius	Muscle; − at belly, + at tendon	M	0.50	0.168
	Muscle; + at belly, − at tendon	M	0.40	0.274
Sartorius	Muscle; − toward recording electrodes	ED	0.90	0.048
	Muscle; − toward recording electrodes	EI	1.40	0.07
	Muscle; + toward recording electrodes	ED	0.70	0.10
	Muscle; + toward recording electrodes	EI	0.35	0.065
Sartorius	Femoral nerve	EI	1.74	0.044
	Muscle, diffuse stimulation			
	Muscle, − in leg	ED	2.90	0.32
	Muscle, − in leg	EI	2.80	0.27
	Muscle, − in abdomen	ED	2.70	0.216
	Muscle, − in abdomen	EI	2.70	0.216

elements have usually a larger k than the more excitable fibers within the same muscle.

Although all these factors influence the measurement of the electrical excitability of both muscle and nerve, it is possible to emphasize a given change more in one than in the other excitable structure. Thus, by proper placing of the electrodes, and a judicious selection of the magnitude of the end-point response, it was possible to produce either increases or decreases of either of the parameters (r or k) of the measured electrical excitability of either the muscle or the nerve fibers supplying it.

In table 2 are given some examples of the marked influence exerted by the relative position of the electrodes with respect to the recording muscle on the excitability parameters. It may be emphasized, on the other hand, that standard experimental conditions yielded reasonably consistent results, as shown in table 3. Furthermore, when several series of observations were made on a given muscle over a period of hours, without any experimental maneuvers other than the anesthesia, the section of the nerve and the insertion of the electrodes, the only change noted in the voltage-capacity curves was a slight progressive upward shift, denoting an increase of the rheobase. This consistency of results in standard conditions justified the study of the changes of excitability after various experimental procedures.

TABLE 3

Values of r and k obtained in different animals

The total resistance of the stimulating circuit was constant. The stimulating cathode was placed in the muscle in the vicinity (1 to 4 mm.) of one of the recording electrodes. The other stimulating and recording electrodes were several centimeters away and in opposite directions. D denotes the direct and I the indirect component of the electrograms.

GASTROCNEMIUS				SARTORIUS			
D		I		D		I	
r	k	r	k	r	k	r	k
0.88	0.066	0.88	0.066	0.86	0.068	0.86	0.068
0.70	0.12	0.70	0.12	1.25	0.10	1.40	0.085
0.76	0.055	0.76	0.055	0.77	0.054	0.77	0.054
1.20	0.09	1.20	0.09	0.62	0.04	0.62	0.04
0.65	0.078	0.65	0.078	0.70	0.10	0.40	0.065

D. *The 4th and 5th stages of neuromuscular transmission.* Two series of experiments were made for testing the changes of electrical excitability of muscles when prolonged stimulation of the motor nerves at 60 per sec. had led to fatigue (4th stage) or to the late rise of tension of the 5th stage. In one series the stimulation of the nerve was stopped at various intervals long enough to construct a voltage-capacity curve, and then renewed. Since recovery from the 4th and 5th stages takes at least 20 to 30 minutes (Rosenblueth and Luco, 1939), and since enough observations could be made in about 5 minutes to show the changes in the curves, such observations supply reliable information for the purpose.

In the second series of experiments the muscles were stimulated directly while the activation of the nerves was continued. The mechanical twitches superimposed on the tetanic curve were employed as indicative of the effectiveness of the condenser discharges. Although the interpretation

of these observations involves several complicating factors, since the results were qualitatively similar to those obtained with the other simpler procedure, it is submitted that the method is adequate to reveal the changes of muscular excitability during the period of stimulation of the nerve.

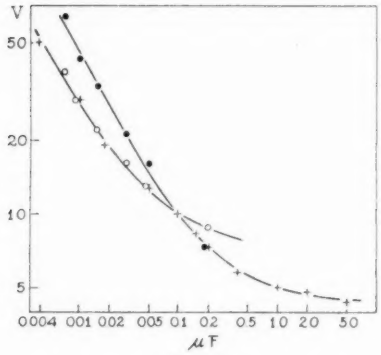

Fig. 3

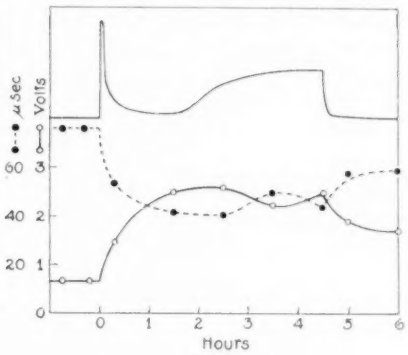

Fig. 4

Fig. 3. Diffuse stimulation of a frog sartorius yielding α and γ voltage-capacity curves. The points indicate the following thresholds: crosses, movement of the recording electrodes on the muscle; dots, "direct" electrical response; circles, "indirect" electrical response. This and the succeeding voltage-capacity curves are plotted in double logarithmic scales. In this instance the scale for the ordinates (volts) was made greater than that for the abscissae (capacities) in order to emphasize the difference between the two curves. The fit with Hill's (1936) formula was excellent for both curves.

Fig. 4. Changes of the electrical excitability of muscle during the 4th and 5th stages. Determinations of r and k were made at the beginning of the experiment for the direct component of the electrograms of gastrocnemius. The popliteal nerve was then stimulated maximally with a frequency of 60 per sec. (beginning at time 0). The stimulation was maintained for 4 hrs. 30 min., with brief interruptions only for the determinations of r and k at the times indicated by the abscissae of the corresponding points. The lowest curve (circles) corresponds to the changes of r; the middle curve (dots) to those of k. Although the tension of the muscle relaxed at each interruption of stimulation and increased upon reapplication, the upper tracing, transferred from the original kymograph record (in conventional units), has been drawn as a continuous line to emphasize the prevailing tension during the 4th and 5th stages. Abscissae: time in hours. Ordinates: r in volts, k in microseconds, and tension.

The results were as follows. The electrical excitability of the muscle decreases progressively as a consequence of prolonged activation of the motor nerve. This decrease is first more rapid, then more gradual, so that a steady level is reached usually during the 4th stage. Further stimulation and the consequent appearance of the 5th stage are not attended by any change of the decreased electrical excitability.

The decreased excitability is due to an increase of the rheobase, not of the time parameter. Indeed, although k may increase slightly after a few minutes of tetanization, it later decreases and is usually less than normal at the steady state in the late 4th and 5th stages. Typical changes of both r and k are illustrated in figure 4. The changes of excitability described are those seen after stimulation of the motor nerve with a frequency of 60 per sec. This frequency is sufficient to elicit marked transmission fatigue (fig. 4). It was possible, however, to observe qualitatively and quantitatively similar effects when the motor nerves were stimulated at frequencies which produce slight or no transmission fatigue (e.g., 30 per sec.).

The decrease of excitability during the 4th and 5th stages is reversible. A sufficient period of rest resulted as a rule in a gradual return to the original curve obtained before stimulation of the nerve. Recovery was fairly prompt (30 min.) after short periods of stimulation (10 to 20 min.). It was slow after prolonged stimulations (fig. 4). The course of the recovery was first rapid and then progressively slower. The initial rapid change was especially noticeable in the experiments in which the excitability was determined during the period of stimulation of the nerve. If stimulation was then stopped the threshold value of a given capacity declined so rapidly that complete curves for the evaluation of r and k could not be constructed for several minutes.

The changes of excitability described were not confined to the muscle but affected also the nerve supply, as shown by similar changes in the curves for both the direct and indirect components of the electrograms. It was considered important to investigate whether a decreased excitability would also be found when the nerve fibers were stimulated in the nerve trunk, instead of in the midst of the muscle. Accordingly, in several experiments two pairs of electrodes were placed on the motor nerve, in addition to those in the muscle. The distal pair on the nerve was used for the tetanic stimulation and the proximal pair for the stimuli from which voltage-capacity curves were plotted. In figure 5 are shown typical results. The curves in A show the excitability changes for both the direct and the indirect components of the electrograms when the muscle was stimulated; the excitability of the two components was practically identical throughout the experiment. The curves in B indicate the changes which took place in the nerve fibers at the trunk. It is apparent that after prolonged stimulation the changes in the excitability of the nerve trunk fibers were qualitatively similar to those which occurred at the muscle.

E. *Curare.* That curare may completely block neuromuscular transmission without any decrease of muscular electrical excitability was seen in 3 out of 8 animals studied (nos. 1–3, table 4). If more curare was then injected into these animals the excitability decreased, as shown by a

large increase of both the rheobase and the time parameter. Figure 6 illustrates a typical instance.

In the other 5 animals examined (nos. 4–8, table 4) a dose of curare just sufficient to paralyze all of the muscle fibers resulted in a decreased muscular excitability (fig. 7).

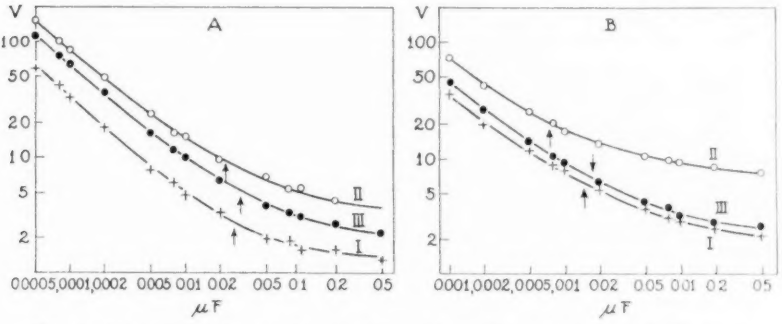

Fig. 5. Changes of electrical excitability of muscle and nerve with fatigue. A, curves for muscle (direct component) and nerve (indirect component) obtained from stimuli applied to gastrocnemius. B, curves for the motor fibers in the popliteal nerve. Curves AI and BI were first constructed. The popliteal nerve was then stimulated at the rate of 60 per sec. for 20 min. through a more distally placed pair of electrodes. Curve BII was constructed from 2 to 8 min., and curve AII from 9 to 17 min. after the end of this stimulation. Curves AIII and BIII were obtained 30 to 40 min. later. The arrows indicate the position of k.

TABLE 4

Changes of electrical excitability of muscle produced by a paralyzing dose of curare

In the animals marked by a * the decurarization took place without any drugs; in those marked by a † the decurarization was produced by prostigmin.

ANIMAL NUMBER	NORMAL		AFTER CURARE		AFTER DECURARIZATION	
	r	k	r	k	r	k
1	0.86	0.068	0.86	0.068		
2	1.25	0.10	1.28	0.075		
3†	1.85	0.071	1.85	0.071	1.85	0.071
4*	2.80	0.046	4.50	0.037	1.90	0.03
5	1.10	0.069	2.30	0.15		
6*	1.25	0.045	1.90	0.14	1.10	0.09
7†	0.88	0.066	2.70	0.15	1.65	0.053
8†	1.40	0.054	1.90	0.15	2.10	0.048

Decurarization, whether produced by prostigmin or by elimination of the curare after some time, usually resulted in an increase of excitability when curare had caused a decrease (fig. 7). Total decurarization could

take place, however, at a time when either the voltage (animals 7 and 8, table 4) or the time parameter (animal 6) were still greater than originally.

Prostigmin. When this drug was injected after curare, in order to produce decurarization, it was found to increase the muscular excitability if this excitability had been depressed by curare. It was interesting, therefore, to see whether prostigmin injected without any previous administration of curare would render the muscles hyperexcitable.

The results were negative. Injections of 0.5 mgm. prostigmin, a dose quite sufficient for decurarization and also for potentiation of the muscular

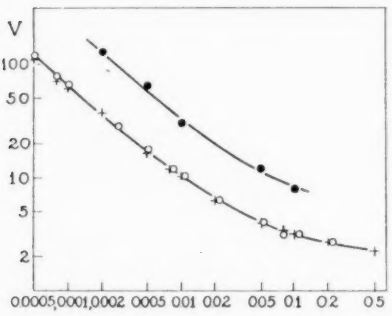

Fig. 6

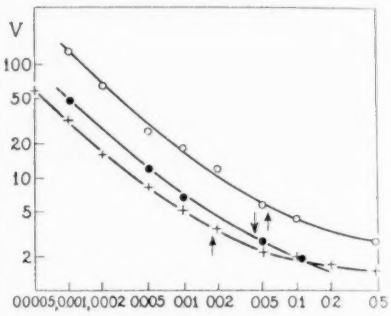

Fig. 7

Fig. 6. Curarization without change of muscular excitability and effect of a further dose of curare (animal 3, table 4). Voltage-capacity curves from direct component of the electrogram of gastrocnemius. The observations marked by the crosses were first made. Curare was then injected and when complete paralysis had taken place the circles were obtained. A further dose of curare shifted the curve to the position of the dots.

Fig. 7. Decrease of muscular excitability with curare (animal 6, table 4). The lower curve (crosses) was constructed from the direct component of the electrogram of sartorius. A dose of curare was then injected which was just enough for total paralysis of the muscle. The upper curve (circles) was then obtained; the animal was still completely curarized. One hour later the curarization had largely disappeared. The middle curve was then constructed.

responses to the motor nerve impulses, had no significant effect upon the muscular excitability.

As was shown by Brown, Dale and Feldberg (1936), eserine (and also prostigmin, Rosenblueth and Morison, 1937a) converts the responses of muscles to single nerve impulses from single twitches to repetitive tetani. It was considered important to find out whether the responses to direct electrical stimulation would likewise become repetitive after prostigmin, or would retain their original simple character.

For this purpose a position of the stimulating electrodes was adopted which would stimulate primarily the muscle fibers, and only activate the

nerve filaments with relatively strong shocks. Typical results are illustrated in figures 8 and 9 for gastrocnemius and sartorius, respectively. In both muscles repetitive responses only take place when the nerves are activated and not when the stimuli act directly. It is obvious, therefore, that the repetitive character of the responses to nerve impulses after prostigmin is caused by some change at the neuromuscular junction, not at the muscle.

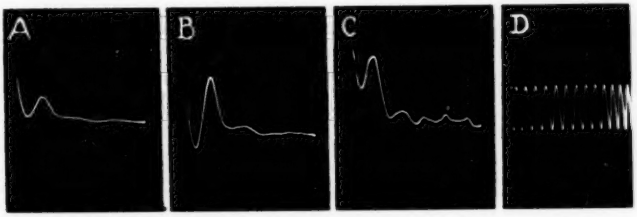

Fig. 8. Diffuse stimulation of gastrocnemius after prostigmin. The capacity of the discharges was the same for A, B and C: 0.1 μF.

A. Voltage 20; pure direct response.

B. Voltage 25; a small indirect response is followed by slight indications of repetitiveness.

C. Voltage 30; marked repetitiveness.

D. 2,000 cycles.

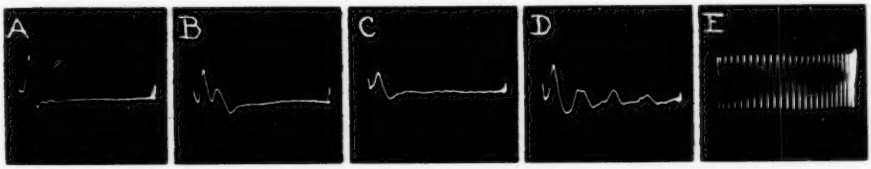

Fig. 9. Diffuse stimulation of sartorius before and after prostigmin. Capacity of the discharges: 0.005 μF.

A and B. Before prostigmin; 40 and 60 volts.

C and D. After prostigmin; 35 and 60 volts.

E. 1,000 cycles.

Discussion. *The method.* The tests described in section A indicate that the prompt responses of muscles to direct stimulation may be readily distinguished from the delayed responses to indirect activation. When both components, direct and indirect, are present in the electrograms it is quite probable that they indicate the stimulation of two different groups of muscle fibers, for the elements activated directly would be refractory at the end of the neuromuscular delay, and would therefore not respond again, even if the corresponding motor nerve fibers had been caused to discharge by the electric pulse.

The previous consideration leads to the inference that if, upon the

progressive intensification of the voltage with which a given capacity is delivered, in certain experimental conditions, the nerve filaments are first stimulated, further strengthening of the shocks may cause the appearance of a direct response in some of the elements which had been already activated indirectly. The voltage-capacity curve for the indirect responses might thus be complicated unless just threshold activity is used as an end point. On the other hand, no such complication can take place in the observations of the direct excitability.

The neuromuscular delay. Neuromuscular delay, like interneuronal synaptic delay, has been frequently attributed to slowed conduction at the fine nerve terminals. The present data (section B) leave no question as to the existence of a true delay after the arrival of the nerve impulse at the muscle and before the appearance of the muscle action potential. Even if the electrical excitability of the nerve fibers should be greater before than after their final terminal branching, sufficiently strong electrical shocks would stimulate directly the last excitable portion of the nerve. In these experiments, marked intensification of the stimuli could decrease slightly the delay recorded with weak shocks but never abolished the interval separating the direct from the indirect components of the electrograms.

It is hardly necessary to emphasize that the neuromuscular delay is strong evidence of the discontinuity in the excitatory process which takes place at the neuromuscular junction, a discontinuity which has only been satisfactorily explained by the theory of chemical transmission of the motor nerve impulses.

The problem of isochronism. The variability of the time parameter k (and consequently of the chronaxie) in different experimental conditions was emphasized in section B. A similar variability has been encountered in previous studies of nerve or muscle electrical excitability (Davis, 1923; Bishop, 1928; Watts, 1924; Lapicque, 1931; Rushton, 1932a and b; Grundfest, 1932; Lambert, Skinner and Forbes, 1933). The voltage parameter r varies likewise considerably in different experimental conditions—e.g., with the interelectrodal distance (Rushton, 1927), the angle between the axis of the electrical field and that of the excited element (Rushton, 1927), the distance between the stimulating electrodes and the end of a cut nerve (Lambert, Skinner and Forbes, 1933), etc. It appears, therefore, that the measurement of the absolute electrical excitability of an element is not feasible; the measurements are always relative to the experimental conditions adopted.

It is true that standard conditions yield satisfactorily consistent results. It is therefore possible to study accurately the changes of excitability in a given tissue and the conclusion of an increase or a decrease of excitability by a given treatment will be thus justified. On the other hand, given the

wide differences in structure and function between nerve and muscle, it is probable that the same experimental procedure would correspond to different stimulating conditions when transferred from one to the other tissue.

In view of these considerations the concept of neuromuscular isochronism or heterochronism is meaningless. Isochronism may be the rule for certain experimental conditions. This was commonly true in the present observations. Rushton's (1933) conclusion that muscles invariably have a longer excitation time than the corresponding motor nerves is therefore not supported by these data. On the other hand, heterochronism may routinely be present when the method of stimulation is favorable for its appearance, as in Rushton's (1932a and b) and Grundfest's (1932) experiments. Lapicque's (1932) assumption that only the procedures which lead to isochronism are valid is arbitrary.

The 4th and 5th stages of transmission. As mentioned in the introduction, the original purpose of these experiments was to find out whether the changes of the muscular electrical excitability during these stages were such that the theory of electrical transmission of the motor nerve impulses could account for the renewal of transmission during the 5th, after the absence of activation during the 4th stage. The data in section D (fig. 4) answer this question negatively. The transition from 4th to 5th stages is not correlated with an increase of the electrical excitability of muscle. Since there is no evidence of an increase in the magnitude of the nerve spike-potentials at this time (Rosenblueth and Luco, 1939) it may be concluded that the electrical theory fails to account for the appearance of the 5th stage.

The changes of excitability with fatigue are in contrast with the negative results reported by Rushton (1933) and in contradiction with the increase of chronaxie suggested by the Lapicques (1919). In any case, because of the increase of rheobase, the muscles are less excitable than normally during the 4th stage. It might be assumed in terms of the electrical theory that the failure of transmission at this stage is due to this hypoexcitability. Since it is possible, however, to decrease fully as much the excitability of the muscles without impairing transmission—e.g., by stimulating at 30 per sec. instead of 60 per sec. (p. 30)—it is apparent that failure of transmission during the 4th stage is not due to the decrease of electrical excitability. There is no evidence of a significant decrease of the nerve spike-potentials when stimulated at the rate of 60 per sec. (Rosenblueth and Luco, *loc. cit.*), a rate which leads to marked transmission fatigue. It may be concluded, therefore, that the electrical theory fails likewise to account for the failure of transmission during the 4th stage.

The discussion of the reversible depression of the excitability of nerve

fibers after prolonged repetitive stimulation (fig. 5) does not belong here, but in a study of nerve fatigability. It is interesting, however, to point out that this hypo-excitability occurs in conditions in which the magnitude of the nerve spike-potential is normal (Rosenblueth and Luco, 1939).

Curare. The data reported in section E (fig. 6) confirm Rushton's (1933) and Grundfest's (1932) observations that complete curarization may take place without any decrease of the electrical excitability of muscle. These data invalidate conclusively the Lapicques' (1908) theory of curarization. Their (1906) observations, that curare may decrease the muscular excitability, are, however, confirmed here (fig. 7). Rushton (1933) attributed such changes to the electrolytes contained in his impure solution of curare. It is very unlikely, however, that 0.1 or 0.2 cc. of a dilute solution of curare injected intravenously in the present experiments could contain a sufficient concentration of electrolytes to account for the marked alterations observed in muscles all over the body.

Since curarization may occur without any change of the electrical excitability of muscles, and since there is no evidence of a decrease of the magnitude of the nerve spike-potentials after curare, it may be concluded that the electrical theory of neuromuscular transmission fails to account for the paralyzing action of curare.

Prostigmin. The fact that prostigmin raises the electrical excitability of muscle after this excitability has been decreased by curare (table 4) might suggest that prostigmin exerts some action directly upon the muscle fibers. This suggestion is not supported, however, by the absence of any change of excitability when prostigmin is administered to normal muscles (section F). It appears likely that the decurarizing action of prostigmin is due to a cancellation of the effects of curare, rather than to an action on muscle opposite in direction to that of curare. For instance, prostigmin might dislodge curare from the chemical or physicochemical combination responsible for paralysis.

Prostigmin not only does not affect the muscular electrical excitability, but, as shown by the observations illustrated in figures 8 and 9, it does not modify the character of the responses to direct stimulation—i.e., these responses are single, not repetitive, unlike those evoked by single nerve impulses. A similar lack of potentiation (repetitiveness) of direct as opposed to indirect responses was shown by Feng and Shen (1937) and by Feng (1937, 1938) after treatment by eserine, barium and guanidine, in frog's muscles. This evidence invalidates Eccles' (1936) suggestion that the potentiation of muscular responses by eserine is due to changes in the reacting muscles.

Since the muscular electrical excitability is normal after prostigmin, and since there is no evidence of any significant change of the nerve spike-

potentials in these circumstances, it may be concluded that the electrical theory of neuromuscular transmission fails to explain the potentiating action of prostigmin on the responses to single nerve volleys.

SUMMARY

The electric responses of muscles stimulated directly are readily distinguished by their promptness from the responses elicited by indirect stimulation (figs. 1 and 2) in which the neuromuscular delay causes a lag (table 1, p. 34). The electrical excitability of innervated muscles stimulated directly could thus be studied independently of that of the corresponding nerve fibers (fig. 3, tables 2 and 3).

The electrical excitability of both nerve and muscle decreased after some minutes (20 to 60) of repetitive stimulation (fig. 5). This decrease took place whether (frequency 60 per sec.) or not (frequency 30 per sec.) neuromuscular transmission fatigue developed (p. 30). The phenomenon was reversible (fig. 5). No significant change of muscular excitability was found when prolonged stimulation at 60 per sec. led to the appearance of renewed transmission (5th stage) following fatigue (4th stage, fig. 4).

Complete curarization could occur without any change of the muscular electrical excitability (fig. 6, table 4). Curare, however, could cause a decrease of excitability (fig. 7, table 4).

Although prostigmin *per se* did not modify the muscular excitability (p. 32), it could cause an increase if injected after curare (table 4). While the responses of muscles to single nerve volleys were repetitive after prostigmin, those to direct stimulation were single (figs. 8 and 9).

The electrical theory of neuromuscular transmission fails to explain the 4th and 5th stages (p. 35), the block of transmission produced by curare (p. 36), and the repetitive character of the responses to nerve volleys after prostigmin (p. 36).

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DISTURBANCES IN REPRODUCTIVE FUNCTIONS CAUSED BY HYPOTHALAMIC LESIONS IN FEMALE GUINEA PIGS[1]

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It is known that hypophysectomy results in genital atrophy. Smith (1930) has shown that this is caused by removal of the anterior lobe independently of any associated brain injury, because of the striking restoration effected by replacement therapy. Nevertheless atrophy of the genitals may follow the placing of small lesions in the hypothalamus even though this is approached by the temporal route which should reduce the danger of direct damage to the hypophysis (Bailey and Bremer, 1921; Biggart and Alexander, 1939). Section of the hypophyseal stalk has produced varying amounts of disturbance in the structure and function of the genital organs depending on the animal used and the investigator doing the work (Richter, 1933; Mahoney and Sheehan, 1936; Brooks, 1938; Westman and Jacobsohn, 1938; Dempsey, 1939). Female cats with small lesions in the hypothalamus behind the optic chiasma, interrupting the hypothalamico-hypophyseal tract and causing diabetes insipidus, were never observed to come into heat and never bred in the laboratory (Fisher, Magoun and Ranson, 1938). From these few references to the literature it is apparent that there is no uniformity of opinion concerning the control which the hypothalamus may exert over reproductive functions nor as to whether this control is exerted through the hypophysis or through some other channel. The present investigation has been directed toward a solution of these problems.

PROCEDURE. A series of 74 female guinea pigs, weighing 400 to 500 grams, were observed for at least 2 weeks at the beginning of the experiment to determine the length of the cycle and the approximate time of opening of the vaginal membrane. One group of animals, 40 in number, was placed with males and then operated during various stages of pregnancy, while the second group, 34 in number, was operated before mating.

The operation was carried out under sterile conditions. The animals

[1] This work was begun under a grant from the Committee on Research in Endocrinology of the National Research Council for a study of the relation of experimental diabetes insipidus to dystocia and has been continued under a grant from the Committee for Research in Problems of Sex of the National Research Council.

were anesthetized by a subcutaneous injection of pentobarbital sodium. Lesions were aseptically placed in the hypothalamus with the Horsley-Clarke instrument and unipolar electrodes. An attempt was made to locate the damage across the midline at the ventral surface of the brain between the optic chiasma and the pituitary stalk, and while there was some slight variation in position macroscopic examination of the brains at autopsy showed that the placement had been successful in almost every instance. Three punctures were made, one in the midline and one a millimeter on either side of the midline; and in each case the electrode was lowered until the base of the skull was reached and then it was lifted 1 mm. and a lesion was made by passing a current of 3 milliamperes for 30 seconds. The bare tip of the insulated nichrome wire in the brain was the anode; and a large indifferent electrode in the rectum served as the cathode. The three lesions only 1 mm. apart fused across the midline. The wounds were closed with skin clips and the animals were kept in an incubator for 24 hours and thereafter at room temperature.

At various periods during the course of the experiment the operated animals were placed in metabolism cages and the water intake was recorded. It was found that a large percentage of the animals developed diabetes insipidus.

Following the operation the majority of the pregnant animals either aborted or resorbed their fetuses and were thereafter treated exactly like the virgin pigs. All of these animals whether virgin or not were separated into groups, about 6 to a group and a male was placed with each group. At frequent intervals the males were shifted from one cage to another or replaced by fresh males. Daily observations were made of the vaginal membranes.

It soon became evident that the great majority of the animals in both of these groups were sterile as judged by the fact that they failed to become pregnant although they were kept with males for a number of months. They showed obvious sexual disturbances and according to the nature of these disturbances they could be classified into 4 groups: 1, animals with vaginal membrane continuously open; 2, animals with the membrane constantly closed; 3, animals which ran regular cycles so far as opening and closing of the membrane was concerned but which failed to become pregnant, and 4, animals which ran irregular cycles, including some which remained open most of the time but occasionally closed and others which remained closed most of the time but occasionally opened.

The sterility might be due to a failure to ovulate, refusal to mate or to both combined. It was, therefore, necessary to determine whether mating occurred. Since estrus in the guinea pig occurs almost exclusively at night, accurate information concerning it can be obtained only by hourly observation throughout the 24 hours. The activities of the female during

proestrus and estrus as described by Young, Dempsey and Myers (1935) are characteristic and serve as a reliable index of sexual drive.

Twelve normal animals were first run as controls in order that we might become acquainted with the nature and regularity of the estrous reflex. The animals were placed in the observation cages about 2 days before the membrane was expected to open, as determined by previous daily examination over a period of several cycles. Hourly observations throughout the day and night were made for proestrous activity. At the first signs of such activity or if the vaginal membrane opened before this activity was observed the animals were at hourly intervals stroked over the back or vulvar region to elicit the estrous reflex or were placed with a male to see if mating would occur. After the reflex had been observed the animal was placed with the male at hourly intervals.

The sterile animals which ran regular cycles were observed in a similar manner except that since they usually showed no proestrous activity they were stroked every hour during the period that the vaginal membrane was open in an attempt to elicit the estrous reflex. These animals were observed in this manner during 2 cycles.

The procedure had to be modified for the animals which were constantly open or closed or which ran irregular cycles. The first two groups were watched continuously for a period of 19 days in the case of the former and a period of 15 days in the case of the latter, observations being made at hourly intervals for proestrous activity and the effect of stroking the back or vulvar regions. In the one instance in which a positive estrous reflex was obtained the female was placed with a male and copulation observed. The pigs with the irregular cycles were placed in the cages and observed in the same manner except that they remained in the cages until the vaginal membrane either opened or closed.

After these observations had been made the pigs with regular and those with irregular cycles were killed at various stages in the cycle but usually three days after the membrane had opened. The animals in which the vagina remained always open or always closed were killed at any convenient time. After the pigs were killed with ether the head was perfused through the arteries with formalin, and the brain with hypophysis attached was removed and placed in formalin. The ovaries, adrenals, thyroid, uterus and vagina were fixed in Zenker's fluid. Both ovaries from each pig have been embedded in paraffin, cut into serial sections 10μ thick and stained with hematoxylin and eosin. The hypothalamus with hypophysis attached was removed from each of the brains, embedded in paraffin, cut into serial sections and every tenth section stained with cresyl violet.

Results. Of the 40 animals operated while pregnant 11 died. Five of the surviving animals became pregnant again. The remaining 24 animals

were regarded as sterile since they failed to become pregnant again although they were kept with the males for a number of months. Only 8 of the 34 virgins subjected to operation became pregnant leaving 26 which were apparently sterile.

All these 50 sterile pigs will be considered together since the previous pregnancy seemed to have no influence on the character of the sexual disturbances resulting from the hypothalamic lesions. According to the nature of these disturbances these pigs may be separated into the four groups already mentioned. Before attempting to study the mating activity in these animals observations were made on a dozen normal females. We were able to observe proestrous activity and elicit the estrous reflex in every one of the normal animals. Copulation was observed in 11 of the 12. The one exception was a pig which went through heat before the vaginal membrane opened.

Animals in which the vagina was always open. There were 12 pigs with vagina always open and with greatly enlarged external genitalia. Three of these animals had been operated while pregnant and the remainder while still virgins. The group as a whole appeared quite healthy, although they weighed less than those of the other two groups and the majority of them were extremely active and developed rough coats. Vaginal smears were made for a number of weeks and showed a very erratic picture. Leucocytes and cornified cells could always be found in the field, although the percentage of each varied from time to time.

Hourly observations for mating activity were made on 8 of these animals for a period of 19 days, the other 4 having been sacrificed before such observations were started. Of these 8, 5 failed to show any evidence of heat, while 2 showed proestrous activity only and 1 showed proestrous behavior and the estrous reflex and copulated.

It was found upon sacrificing these animals that the uterus and vagina were extremely enlarged as is shown in figure 1, A. Adhesions of the uterus and vagina were found in several instances.

Microscopic examination of the ovaries revealed many well developed follicles. Although these animals were all killed without reference to any cycle, since none could be detected, the ovaries were all in a state of follicular development corresponding with that of ovaries from normal animals killed in the last half of the cycle. However, the ovaries as a whole appeared to be more cystic than the normals. The most striking abnormality was the almost complete absence of corpora lutea. Only in two animals were these bodies found and in both instances the corpora lutea appeared to be old and undergoing degeneration.

Animals in which the vagina was always closed. The 7 animals of this group had all been operated while pregnant. They all were healthy and had a sleek, well groomed appearance. They were, however, less active

than those of the previous group and gained more rapidly in weight. At the termination of the experiment they averaged about 250 grams heavier than the animals in which the vagina was always open and were equal in weight to the animals which ran regular cycles.

Observations for mating activity were extended throughout 15 days and no signs of sexual interest were noted. None of the animals showed proestrous activity and in none of them could an estrous reflex be elicited.

It was found upon sacrificing the animals that the uterus, vagina and ovaries were atrophic (fig. 1, B). On microscopic examination the ovaries showed a complete absence of large follicles and also an absence of corpora lutea. Large numbers of primary follicles could be found at the periphery of the ovary. There appeared to be more of these present than in normal ovaries but they may have been merely more closely packed together due

A B C

Fig. 1. Uteri from three guinea pigs: A, from one with vaginal membrane always open; B, from one with vaginal membrane always closed; C, from a normal guinea pig which had been pregnant several months previously and which was killed while the vaginal membrane was open.

to the absence of large follicles and the resulting small size of the ovary. The largest follicles were about the size of those usually encountered during the first few days of the estrous cycle in the normal animal. Numerous follicles in which antrum formation had started could be found and many of these were undergoing atresia.

Animals which ran regular cycles. A total of 23 pigs ran apparently normal cycles but failed to become pregnant although they were kept with males for a considerable time. Nine of these animals had been operated while pregnant and the remainder while still virgins. They did not differ noticeably from normals in appearance, weight or activity.

Nineteen of these animals were observed hourly for mating activity during that portion of 2 cycles during which the vaginal membrane remained open. Eleven of these showed neither proestrous nor estrous ac-

tivity. Five animals showed some slight indications of proestrous during one of the periods of observation and were negative the next, but no estrous reflex could be elicited from them. Two animals showed proestrous activity during both periods but failed to show estrous activity. Only one pig gave the estrous reflex and copulated. This was during the second cycle under observation and this same pig had been entirely negative during the first cycle.

Out of the entire series of 74 operated pigs 13 became pregnant after the operation. These animals ran regular cycles before becoming pregnant and in at least one of these cycles must have accepted the male. It is not known whether or not they resembled the pig mentioned last in the preceding paragraph and showed estrous behavior during some of the cycles and were refractory during others.

The animals of this group were sacrificed at various stages of the estrous cycle but the largest number were killed 3 days after opening of the vaginal membrane. The uterus, vagina and ovaries resembled those taken from normal animals during a corresponding period of the cycle except that a somewhat larger number of the ovaries appeared to be cystic. Serial sections revealed follicular development comparable to that of normal ovaries at the same stage in the cycle. Normal corpora lutea could be found in all of the ovaries, and those in the ovaries removed from the pigs killed at about the time of closure of the vaginal membrane were fresh corpora lutea.

Animals which ran irregular cycles. Of the 8 animals in this group 5 were operated while pregnant and 3 while still virgins. They may be subdivided into the following subgroups:

1. There were 2 animals in which the vagina remained closed for a much longer time than normal but would then open up and remain open for the normal length of time. Both had the same general appearance as those in which the vagina remained continuously closed. They were killed while the vaginal membrane was open and the ovaries contained newly formed corpora lutea and a few large follicles, but otherwise they showed a poor follicular development and resembled those of the animals in which the vaginal membrane was always closed.

2. In 2 animals the vagina remained open most of the time although it did close occasionally. These animals appeared quite similar to those in which the vaginal membrane was always open. They were sacrificed after having been open for many days and the vagina and uteri were greatly hypertrophied. A microscopic examination of the ovaries revealed that they were similar to those found in the animals that were always open, i.e., there was an absence of corpora lutea and a high degree of follicular development.

3. Three animals ran cycles of irregular length and after opening remained open for variable periods of time. One was sacrificed 3 days after the membrane opened and the ovaries were histologically normal. The other 2 were killed after the membrane had been closed for 13 and 36 days respectively and both showed an absence of mature ovarian follicles and one also an absence of corpora lutea.

4. The vaginal membrane of the remaining animal of this group was open for a considerable length of time following the operation. Approximately six weeks before the termination of the experiment, the membrane closed, and in the intervening period tears appeared in the membrane at regular intervals but in no instance did it open completely. The uterus and vagina were found to be greatly hypertrophied upon sacrificing the animal. No microscopic study of the ovaries has been made.

Diabetes insipidus developed in many of these guinea pigs but no regular relation could be detected between the disturbance in water metabolism and the abnormalities of reproductive functions. In classifying the animals according to their consumption of water we rated the animals with a daily intake of 50 to 90 cc. of water as having a mild polydipsia, 90 to 150 cc. a good polydipsia and over 150 a severe polydipsia. Of the 12 animals with vagina continuously open 7 showed a polydipsia (4 mild and 3 good). All 7 of the animals with vagina continuously closed showed polydipsia (2 mild and 5 good). Of the 23 animals that ran regular cycles 16 showed polydipsia (8 mild, 3 good and 5 severe). All but one of the animals which ran irregular cycles showed a polydipsia (3 severe, 3 good and 1 mild).

The hypophysis. An examination of 10μ sections of the hypophysis, stained with cresyl violet, showed that atrophy of the pars nervosa had resulted from the interruption of the supraoptico-hypophyseal tract in the hypothalamus. In only 3 of the hypophyses were definite lesions of the anterior lobe detectable. This excludes the possibility that direct damage to the hypophysis was responsible for any of the symptoms observed.

Discussion. The continued growth of the guinea pigs after the operation speaks against a deficiency in hypophyseal growth hormone. The animals with continuously open vaginae, hypertrophied uteri, and ovaries showing a high degree of follicular development without ovulation, probably had a continuous and perhaps excessive secretion of estrin (Nelson, 1939) which may perhaps have been due to a deficiency in the luteinizing hormone from the anterior lobe of the hypophysis. The animals with vagina continuously closed and with atrophic uteri and ovaries resembled somewhat hypophysectomized animals although there was no gross damage to the hypophysis and no retardation of body growth.

In the animals which ran regular cycles so far as the changes in the vagina and ovaries were concerned there is every reason to believe that estrin and

progesterone were being formed normally. But these animals did not come into heat, show proestrous or estrous behavior and would not accept the male. Since these ovarian hormones will induce estrus in spayed guinea pigs (Collins, Boling, Dempsey and Young, 1938; Boling, Young and Dempsey, 1938) one must take into consideration the possibility that the lesions may have seriously disturbed the neural mechanisms for the mating reactions.

SUMMARY

In female guinea pigs lesions in the hypothalamus between the optic chiasma and the attachment of the infundibular stalk caused sterility and disturbances in reproductive functions on the basis of which the animals could be classified into 4 groups:

1. Animals with vagina continuously open and with enlarged uteri and follicular ovaries without corpora lutea. These failed with one exception to show estrous behavior.

2. Animals with vagina continuously closed, atrophic uteri and small ovaries, showing a complete absence of large follicles and of corpora lutea. These all failed to show estrous behavior.

3. Animals in which the vagina opened and closed in cycles of normal length and in which the ovaries had normally well developed follicles and corpora lutea. These with one exception failed to show estrous behavior.

4. Animals in which the vaginal membrane opened and closed at irregular intervals. Some of these animals resembled those of the first group and others resembled those of the second group.

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RESPIRATORY ADJUSTMENTS TO OXYGEN-LACK IN THE PRESENCE OF CARBON DIOXIDE

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While Haldane and Priestley (1905) demonstrated the close dependence of respiratory volume on alveolar CO_2 concentration when the oxygen supply is adequate, the effects of high CO_2 in the presence of low oxygen are not so well known. In this paper it is proposed to describe quantitatively some of the respiratory responses of two normal human subjects to various proportions of CO_2 and oxygen in inspired air. The data will be interpreted in the light of current hypotheses regarding respiratory regulation.

EXPERIMENTAL PROCEDURE. The subject reported in the morning without breakfast and rested on a bed for about one-half hour before the experiment began. Suitable proportions of air, nitrogen, and CO_2 were mixed in a gasometer of about 600 liters' capacity and samples taken for analysis. The subject breathed from this gasometer and expired into a smaller gasometer on which readings were taken every minute. From these readings the ventilation in liters per minute at standard conditions was calculated. A blood sample was drawn from the brachial artery towards the close of many experiments: one portion was analyzed on the Van Slyke apparatus for total CO_2 and O_2 content; another was equilibrated with CO_2 and O_2 at 37°C. according to the method described by Dill, Graybiel, Hurtado, and Taquini (1940) in order to determine the oxygen and CO_2 combining capacity of the blood and to calculate the pCO_2 (partial pressure of CO_2) and pH of arterial serum.

SUBJECT	AGE	HEIGHT	WEIGHT	B.M.R.
	yrs.	*cm.*	*kgm.*	*per cent*
P	24	174	61.3	−2
Z	21	179	77.2	−12

Some of the gross physical characteristics of the two subjects are listed in the accompanying table. P. had been a well-trained distance runner at college a few years previously but was not in active training at the time of

the experiments. Both subjects were in good health. On subject P. four to six experiments were run in each series with rest periods of about 15 minutes intervening. Usually the first experiment on P. was with atmospheric air and the most severe experiment was left to the last of the morning. The experiments on P. extended over more than a month, from November 9 to December 16. That the subject maintained a constant physiological state is doubtful; some of the experiments indicate day-to-day variation. The experiments on subject Z. were conducted over a shorter period of time, from December 19 to January 5 (except for a few

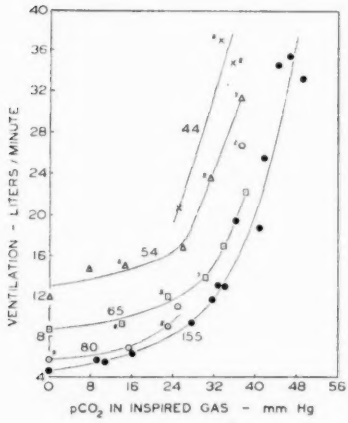

Fig. 1

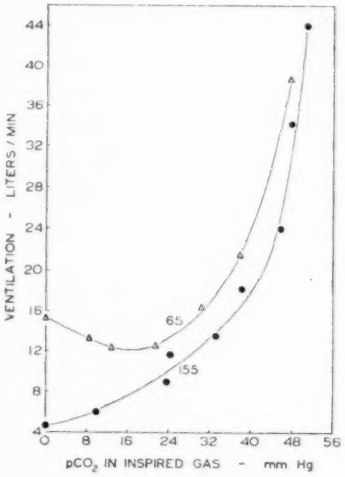

Fig. 2

Fig. 1. Total ventilation of subject P. in relation to pCO_2 and pO_2 in the inspired gas. The pO_2 values are 44, 54, 65, 80 and 155 mm., as shown along the curves. Some of the points correspond to the averaged results of two or more experiments, the number being indicated in the figure.

Fig. 2. Total ventilation of subject Z. in relation to pCO_2 and pO_2 in the inspired gas. The pO_2 values are 65 and 155 mm.

introductory experiments), and were limited in number to not more than two each day. Possibly because of these precautions more consistent data were obtained on him than on P.

Influence of CO_2-excess and of O_2-lack on ventilation. Ventilation in liters per minute is plotted against the pCO_2 in the inspired air for subject P. in figure 1 and for subject Z. in figure 2. Each value for ventilation is the mean of the last 5 minutes of each experiment, except when the ventilation increased or decreased during the arterial puncture; in such cases the mean value for the 5 minutes preceding the puncture was used.

It will be noted that many of the points correspond to the averaged results of two or more experiments in which conditions were nearly the same.

The additive nature of the response to O_2-lack and CO_2-excess is clear: for any given pCO_2 the increase in ventilation depends upon the degree of O_2-lack, and with increasing concentration of CO_2 the ventilation increases more and more rapidly. Within limits, the lower the pO_2 of inspired air the greater the effect upon ventilation of a given change in the pO_2. In severe anoxia, however, the response is less predictable. The interpretation of these observations depends in part on the S-shape of the dissociation curve of oxyhemoglobin: a given small decrease in pO_2 reduces the percentage saturation in the steep part of the curve more than in the flatter part. Furthermore, a given reduction in saturation is more disturbing to an organism already suffering from oxygen-lack in tissues than to an organism having the usual oxygen supply. As suggested above, this holds only within limits; eventual breakdown may occur either through excessive alkalosis from loss of CO_2 or metabolic upsets from lack of oxygen, or both.

Qualitatively the responses of the two subjects were alike, but consistent quantitative differences appeared as soon as the oxygen pressure reached uncomfortably low levels. This is in line with the Henderson-Prince rebreathing tests formerly used to classify candidates for high altitude flight. The stage of anoxemia at which breathing became irregular or decreased abruptly in volume varied from one subject to another.

Subject Z., after consciously experiencing symptoms of O_2-lack, became semi-comatose during the last minutes of tests in which anoxemia was greatest. Ventilation then suddenly increased and was maintained for some minutes at the higher level. How can we explain this sudden increase of ventilation? Is the demand for oxygen likely to overbalance the demand for acid-base regulation? Is lactic acid released into the respiratory center in extreme anoxemia? Whatever the answers to these questions may be, it seems reasonable to suppose that, just as there exist wide species differences with respect to tolerance of CO_2 accumulation or of CO_2-lack (cf. diving animals, on the one hand, and panting animals, on the other), so among men some are relatively tolerant of the alkalosis associated with anoxia and by sustained overventilation withstand unusually low partial pressures of oxygen in inspired air. Overventilation maintains a small differential pressure between inspired air and alveolar air and alkalosis favors the uptake of oxygen by hemoglobin. This argument is supported by the observation of Rice and Steinhaus (1931) that the dog is notably tolerant of alkalosis and McDonough's demonstration (1939) of the dog's unusual resistance to oxygen-lack: they can survive an exposure of several hours to an atmosphere having a pO_2 of 45 mm.

Regulation of pH. The effect of various combinations of pCO_2 and pO_2

on the pH of arterial serum is shown in figure 3. A series of straight lines can be drawn through the several sets of points, all of which come near the point $pCO_2 = 38$ and pH = 7.37. Whatever the degree of oxygen-lack, a pCO_2 of 38 in inspired air means that arterial blood has its normal reaction. It is also worth noting that when normal saturation is insured by a high pO_2 (155 mm.) the pH of arterial blood remains unchanged through a wider range of pCO_2. It is difficult to reconcile the theory that a simple relation exists between arterial pH and pulmonary ventilation with the foregoing observations.

It has been proposed at various times that the unpleasant effects of oxygen-lack may be lessened by adding CO_2 in moderate concentrations.

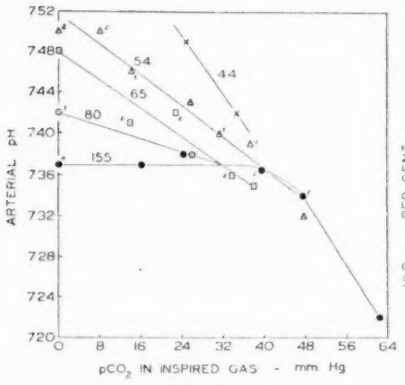

Fig. 3

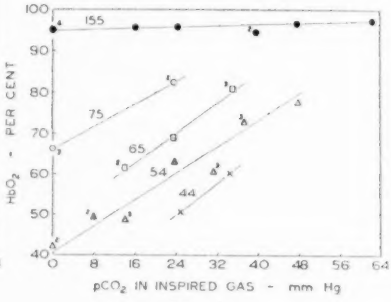

Fig. 4

Fig. 3. Arterial pH in relation to pCO_2 and pO_2 in the inspired gas for both subjects. The number of experiments averaged for each point is indicated.

Fig. 4. Percentage saturation of arterial blood in relation to pCO_2 and pO_2 in the inspired gas for both subjects. The number of experiments averaged for each point is indicated.

The foregoing figure illustrates the advantage gained in maintenance of pH and it is natural to suppose that the increased ventilation raises the saturation of arterial blood. The magnitude of this effect is shown in figure 4. It appears from the curves shown, for example, that in the absence of CO_2 lowering the pO_2 of inspired air from 75 to 54 mm. will lower the arterial saturation from 66 to 41 per cent, but that if 32 mm. of CO_2 are added to the 54 mm. of oxygen in the inspired air, the arterial saturation will rise again from 41 to 66 per cent.

The following ratios compare roughly the effects on arterial oxygen saturation of increments in pCO_2, on the one hand, and of O_2, on the other:

HbO_2, PER CENT	80	70	60
$\frac{\Delta\ pCO_2}{\Delta\ pO_2}$	−1.4	−1.5	−1.1

This means, for example, that if the oxygen saturation is to remain at 80 per cent, a decrement in pO_2 of 10 mm. calls for an increment in pCO_2 of 14 mm. It follows that within the range covered by these observations the arterial saturation will be raised more by a given increase in pO_2 than by an equal increase in pCO_2. This is in qualitative agreement with the report of Jongbloed and Wildschut (1938) that at about one-half an atmosphere adding 8 per cent O_2 raised the arterial saturation nearly twice as much as adding 8 per cent CO_2.

Discussion. In an atmosphere deficient in oxygen respiration is controlled as though stimuli depending on oxygen-lack were opposed by inhibition arising from alkalosis. It is suggested that a given degree of anoxia provides a greater stimulus in one man than in another and that the inhibitory forces arising from a given degree of alkalosis also show individual differences in strength. Such an animal as the dog, which is accustomed to the alkalosis associated with panting, tolerates lower oxygen tensions in inspired air than man. Nielsen and others have found that in so doing the dog maintains a considerable degree of alkalosis without the interruptions seen in man that we call Cheyne-Stokes breathing (1940).

In experiments such as ours blood lactate does not increase and hence when the pCO_2 of inspired air did not exceed 38 mm. the arterial pH was normal or alkaline. The burden of proof is on those who assert that under such circumstances, even though lactic acid does not appear in the blood, it may accumulate in tissues responsible for respiratory regulation, or who postulate some other mechanism by which acidosis may be created in respiratory centers even though alkalosis develops in the blood. While Jacobs (1920) has shown that a condition of intracellular acidity can be produced by a slightly alkaline solution of CO_2 in $NaHCO_3$, this depends in part on the ready movement of CO_2 through cell membranes. It may also depend on a very slow movement of the bicarbonate ion through cell membranes. When acid is being produced within a cell, reaction with bicarbonate and escape of CO_2 presumably maintain the usual gradient of hydrogen ion concentration across the cell membrane.

The ability of diving animals to hold the breath is another illustration of respiratory regulation in which carbon dioxide elimination seems to be subordinated to oxygen supply. Many have had difficulty administering volatile anesthetics to turtles because after the first breath they are apt to suspend respiration for 10 or 15 minutes. Some years ago Dill and

Edwards (1931) found it possible to measure the metabolism of a crocodile by introducing a measured volume of gas through a tracheal cannula into the emptied lungs and after 10 minutes removing, measuring, and analyzing the residual gas. Respiratory distress was not evident as carbon dioxide accumulated, appearing only when the oxygen tension in the lungs reached a low level. Other studies of respiration in diving animals have been reviewed by Irving (1939). They leave no doubt that diverse species of such animals either do not experience or can suppress the stimuli arising from accumulating carbon dioxide.

From other sources also comes evidence that respiratory regulation is less simple than many have supposed. Schmidt, Dumke and Dripps (1939) have questioned some of the rôles assigned to the carotid sinus by the Heymans school. Pitts, Magoun and Ranson (1939) have advanced good evidence that there are two respiratory centers in the brain, one governing inspiration, which they call the inspirato-inhibitory center, and the other governing expiration, the expiratory center.

SUMMARY

The regulation of respiration and the properties of the blood have been studied in two healthy young men exposed to atmospheres deficient in oxygen and containing various concentrations of CO_2. There were significant differences between the two subjects, as is shown by the graphical description of their respiratory responses (figs. 1 and 2).

The addition to inspired air of carbon dioxide renders a given low pO_2 more tolerable, not only on account of an increased arterial saturation but because the disturbance of acid-base balance is reduced. However, the gain in oxygen saturation is less than results from an equal increase in pO_2, the pCO_2 remaining constant.

The bearing of these and related investigations on current theories of respiratory regulation is discussed.

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THE MODIFICATION OF THE HEMODYNAMIC EFFECTS OF ACETYLCHOLINE BY ERGOTAMINE

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The ergot alkaloids, ergotoxine and ergotamine, reverse the pressor effect produced by splanchnic stimulation (1) and by the administration of epinephrine (1 to 8 and others), but not the pressor phenomenon seen after hepatic nerve (4, 6) and lower abdominal sympathetic chain stimulations (4). The modification of the pressor response to epinephrine by ergotoxine and ergotamine has been ascribed to a sympatholytic action (1, 9) but the existence of such an action has recently been questioned by Koppanyi et al. (5, 10, 11, 12). These workers did not observe any depression of the vasoconstrictor sympathetic nerve endings by these alkaloids. They suggested that the reversal of the epinephrine pressor response was not due to paralysis of the sympathetic endings by the ergot alkaloids but to a sensitization of the inherent vasodilator action of epinephrine, since the vasodilator effect of epinephrine was opposed by atropine and enhanced by physostigmine or prostigmin.

Acetylcholine produced in atropinized animals vasopressor and other sympathetic responses presumably by stimulation of the vasoconstrictor ganglia causing liberation of "sympathin" (13, 14) and perhaps of epinephrine (15, 16). These effects on blood pressure are similar to those obtained by the administration of epinephrine or by stimulation of the preganglionic fibers of sympathetic nerves. Hunt (2) assumed that acetylcholine in large doses stimulated both the vasoconstrictor and vasodilator sympathetic ganglionic cells. In one atropinized animal, he observed that ergotoxine changed the pressor response to acetylcholine into a fall in blood pressure. In explaining this phenomenon, he accepted Dale's view (1), namely, that ergot alkaloids depressed the vasoconstrictor but not the vasodilator sympathetic terminations.

The following experiments were carried out to determine the cause of the ergotamine reversal of the acetylcholine pressor effect, and to ascertain whether the action of ergotamine in this respect was qualitatively similar to that of the nicotine reversal of the acetylcholine pressor effect previously described (13).

EXPERIMENTAL. Dogs and cats, anesthetized with nembutal or in a few instances with sigmodal sodium[1] (secondary amyl bromallyl barbituric acid), urethane or ether, were used in these experiments. All drugs were dissolved in physiological saline and administered intravenously unless otherwise stated and the doses expressed in terms of milligrams per kilogram of body weight.[2] The blood pressure was recorded from the right common carotid artery except in the interrupted cross circulation experiments, the technique for which is described in the text. Artificial respiration was routinely applied just prior to the administration of ergotamine tartrate (1:1000 solution) because this drug impairs respiration in many animals. To permit the maximum action of ergotamine about ten minutes were allowed to elapse between the administration of ergotamine and other drugs.

The reversal of the acetylcholine pressor effect by ergotamine. Physostigmine salicylate or prostigmin increases the effectiveness of acetylcholine about ten times; therefore, the amounts of acetylcholine producing comparable rises in blood pressure were correspondingly smaller in the animals of groups II, III and IV of table 1 than those of group I. These pressor responses to acetylcholine chloride in the presence of atropine sulfate alone or of atropine and physostigmine ranged from 9 to 130 mm. of mercury.

Following 0.5 mgm. of ergotamine tartrate, the pressor responses to acetylcholine were converted into pure depressor effects in a few animals, but usually biphasic responses resulted. Additional ergotamine tended to change these biphasic responses to acetylcholine into pure depressor effects, as may be seen in figure 4. In most experiments, a single injection of 1.0 mgm. of ergotamine was employed. The results summarized in table 1 show that after 1.0 mgm. of ergotamine, acetylcholine produced more pure depressor than biphasic responses.[3] In general, complete reversals of the acetylcholine pressor effect by ergotamine were obtained in animals receiving smaller amounts of atropine (figs. 1 and 3) but they may occur even in dogs after as much as 4.0 mgm. of atropine (fig. 4). The depressor effects of acetylcholine following ergotamine varied from 10 to 85 mm.

The pressor responses to epinephrine were not reduced when the pressor

[1] Kindly supplied by Riedel de Haen, Inc.

[2] The term "per kilogram of body weight" will hereafter be omitted to avoid repetition.

[3] The term "biphasic response" to acetylcholine refers to a sequence of blood pressure responses in which a depressor effect precedes a rise in blood pressure. In general, a biphasic response occurring *before* the administration of ergotamine is characterized by slight depressor effects followed by marked pressor responses, whereas *after* 1.0 mgm. of ergotamine large blood pressure falls usually precede smaller rises.

effects of acetylcholine were completely reversed by ergotamine (see fig. 1, V). Two of the nembutalized dogs included in group II of table 1 were adrenalectomized. In these animals, the pressor effects of acetyl-

TABLE 1

The reversal of the acetylcholine pressor effect by ergotamine

ANIMAL	ANESTHETIC*	NUMBER OF ANIMALS	DOSE OF			BLOOD PRESSURE RESPONSE TO ACETYLCHOLINE	DOSE OF ERGOTAMINE	BLOOD PRESSURE RESPONSE TO ACETYLCHOLINE FOLLOWING ERGOTAMINE	
								Number of animals	
			Atropine	Physostigmine‡	Acetylcholine			Depressor	Biphasic†
Group I									
			mgm./kgm.	*mgm./kgm.*	*mgm./kgm.*		*mgm./kgm.*		
Dog	N	3	2.0–3.0		0.25–1.0	Pressor	1.0	2	1
Dog	S	1	1.0		0.1–0.2	Pressor	1.0	1	
Cat	N	3	3.0–5.0		0.3–1.0	Pressor	1.0	1	2
Cat	N	2	3.0–5.0		0.5–1.0	Biphasic†	0.5–1.0	2	
Group II									
Dog	N	10	1.0–5.0	0.5–1.0	0.025–0.1	Pressor	1.0	7	3
Dog	N	1	1.0	1.0	0.1	Pressor	0.6	1	
Dog	S	3	0.25–2.5	0.5–1.0	0.025–0.05	Pressor	1.0	2	1
Cat	N	3	3.0–5.0	0.5	0.05–0.1	Pressor	1.0	1	2
Cat	U	1	2.0	0.5	0.05	Biphasic†	1.0	1	
Group III									
Dog	S	2	0.5–2.5	0.5–1.0	0.0025–0.005	Pressor	1.0	2	
Dog	N	1	2.0	1.0	0.02	Pressor	2.0		1
Cat	N	3	3.0	0.5	0.005–0.01	Pressor	1.0	3	
Group IV									
Dog	U	1	0.5	10.0	0.05	Pressor	1.0	1	
Cat	N	1	3.0	5.0	0.05	Pressor	1.0		1

* N = Nembutal; S = Sigmodal sodium; U = Urethane.

† For explanation, see footnote in text.

‡ Physostigmine or prostigmin in group II.

choline were converted into depressor responses by ergotamine (fig. 3). This indicates that epinephrine liberated from the suprarenal glands by acetylcholine is not a factor in the reversal.

In these experiments, ergotamine itself produced a marked pressor

response in practically all animals, but this pressor effect was reduced or absent on repeated administration (see figs. 3, IX and 4, IV). It also caused slowing of the heart and an increase in pulse pressure in the presence of atropine (see figs. 3, III and 4, II). The slowing of the heart usually ended abruptly within five to eight minutes after ergotamine was administered (fig. 4, II), but the heart seldom resumed its original rate until several minutes more had elapsed and in some animals this rate was never attained. The increase in heart rate and respiratory stimulation from acetylcholine were usually unaffected by ergotamine. This cardiac acceleration was not due to the marked fall in blood pressure because it also occurred when the blood pressure falls were slight.

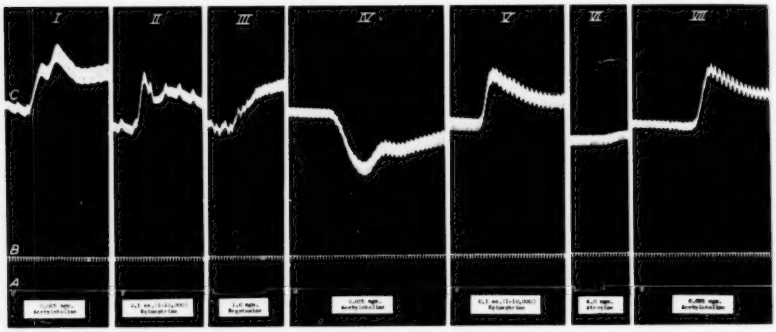

Fig. 1. Showing the ergotamine reversal of the acetylcholine pressor effect and unchanged epinephrine rises after ergotamine.

A, line indicating injection of drugs intravenously. B, time, 1.7 seconds; base line representing zero mm. of Hg pressure. C, blood pressure tracing from the common carotid artery.

Dog, 7.5 kgm., 30 mgm. Nembutal, 1.0 mgm. of atropine sulfate and 1.0 mgm. of prostigmin per kilogram by vein. I. 0.025 mgm. of acetylcholine chloride. II. 0.1 cc. (total) of 1:10,000 epinephrine hydrochloride. III. 1.0 mgm. of ergotamine tartrate. IV. Same as I. V. Same as II. VI. 4.0 mgm. of atropine sulfate. VII. Same as I.

The effect of ergotamine (and nicotine) on the liberation of adrenergic substances by acetylcholine. In these experiments, 20 to 30 cc. of blood were transferred from the left common carotid artery of one dog into the left femoral vein of another by means of a closed system into which a 30 cc. syringe was fitted. The volume of this closed system including the small glass cannulae, rubber-tubing, and three-way Luer syringe stopcock was less than 2 cc. The blood pressure of the donor was taken from a femoral artery and the blood pressure of the recipient from the right common carotid artery. Vasopressor responses were obtained in the nembutalized donors (receiving 3 mgm. of atropine and 1 mgm. of physo-

stigmine) by the injection of 0.05 to 0.1 mgm. of acetylcholine, which presumably stimulates sympathetic ganglia, liberating adrenergic substances. At the beginning of, or about the peak of, the pressor responses in the donor, a definite volume of blood was transferred from the donor into the nembutalized recipient. The recipient received intramuscularly 5 to 10 mgm. of cocaine hydrochloride, which was given to sensitize the response to the transferred adrenergic substances (18, 19, 20), and usually 3.0 mgm. of atropine to nullify the vasodepressor action of any trace of acetylcholine which might be transferred along with the adrenergic substances. Pressor responses in the recipient of less than 1.5 times those of control blood transfers were considered negative.

Some experiments of this nature are not satisfactory due to the incompatibility of the bloods of the two dogs used, to the lack of good pressor responses in the donor, to uncertainty in taking blood approximately at the time of maximum liberation of adrenergic substances or to other unexplained factors. The time of maximum liberation of adrenergic substances is progressively delayed as the donor loses blood and its blood pressure falls, and is more difficult to ascertain when the acetylcholine pressor response, which is the criterion for liberation of adrenergic substances, is reversed by ergotamine.

In four such satisfactory experiments, the blood pressure elevations in the recipients, following transfer of blood within 13 to 20 seconds after acetylcholine was administered to the donors, were one and one-half to more than three times those of control blood injections. After the donors received 1.0 mgm. of ergotamine, which reversed the pressor effect of acetylcholine in these animals, similar transfers following acetylcholine to the donors gave pressor responses in the recipients of almost the same magnitude as those obtained before the administration of ergotamine. These experiments show conclusively that adrenergic substances are liberated in the donors by acetylcholine even in the presence of ergotamine. Acetylcholine itself, which might have been transferred with the blood of the donor, had no influence on the pressor response in the recipient because control injections of acetylcholine into the recipient failed to produce a rise in blood pressure. A typical experiment is shown in figure 2.

In three of the four above experiments, 4 to 6 mgm. of nicotine salicylate were given to the ergotaminized donors and transfers of blood were made following acetylcholine injections as before. The results (fig. 2, IX) show that the pressor responses in the recipients were no greater than those of control blood injections, indicating a lack of liberation of adrenergic substances after nicotine. In three other experiments, nicotine administered to the recipients did not prevent or interfere with the pressor effects of the transferred adrenergic substances.

Direct evidence that ergotamine sensitizes the vasodilator mechanism in reversing the pressor effect of acetylcholine. Non-atropinized animals. In seven of the twenty-three animals under various anesthetic agents of

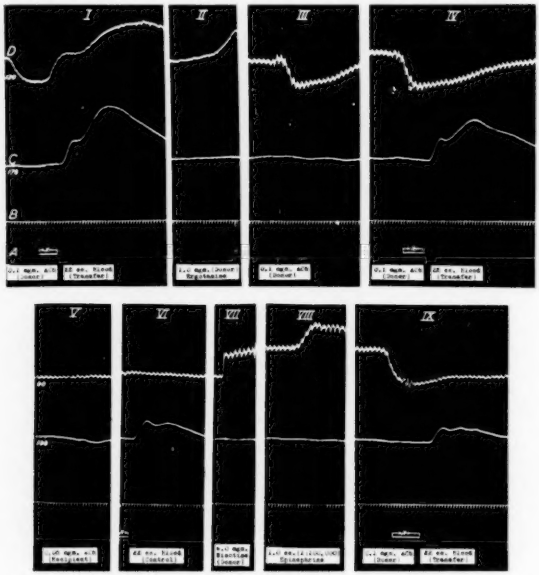

Fig. 2. Showing the transfer of adrenergic substances from one animal to another before and after ergotamine (and before and after nicotine) with controls.

A, line indicating injection of drugs intravenously and the injection of blood into the recipient. B, time, 1.7 seconds. C, blood pressure tracing of the recipient dog from the common carotid artery (mm. Hg pressure indicated numerically). D, blood pressure tracing of the donor dog from a femoral artery (mm. Hg pressure indicated numerically). → x ←, period of taking blood from the donor. Three transfers were made before the first tracing.

Donor dog, 10.1 kgm., 35 mgm. of Nembutal, 3.0 mgm. of atropine sulfate and 1.0 mgm. of physostigmine salicylate per kilogram by vein. Recipient dog, 5.8 kgm., 35 mgm. of Nembutal and 3.0 mgm. of atropine sulfate by vein, and 5 mgm. of cocaine hydrochloride by muscle. I. 0.1 mgm. of acetycholine chloride to the donor, followed by withdrawal of 22 cc. of blood from the donor and injection of the blood into the recipient. II. 1.0 mgm. of ergotamine tartrate to the donor. III. Control injection of acetylcholine into the donor. IV. Same as I. V. Control injection of 0.05 mgm. of acetylcholine into the recipient. VI. Control injection of 22 cc. of blood into the recipient. VII. 6.0 mgm. of nicotine salicylate to the donor. VIII. Control injection of 1.0 cc. (total) of 1:100,000 epinephrine hydrochloride into the donor. IX. Same as I.

group I of table 2, the vasodepressions from very small amounts of acetylcholine were increased by ergotamine and in the others they were decreased or unaffected. (Barium chloride and pitressin also decreased the acetyl-

choline depressor effect in all barbiturized animals, when the doses of these drugs were great enough to produce marked vasoconstriction.)

Atropinized animals. The amount of acetylcholine employed in the animals of group III of table 2 are borderline doses and may produce either vasopressor or vasodepressor responses, whereas pressor responses to the doses of acetylcholine used in group II are obtained in only an

TABLE 2

The effect of ergotamine on the vasodilator response to acetylcholine in non-atropinized and atropinized animals

ANIMAL	NUMBER OF ANIMALS	ANESTHETIC	DOSE OF			EFFECT OF ERGOTAMINE ON THE ACETYLCHOLINE BLOOD PRESSURE FALLS NUMBER OF ANIMALS		
			Atropine	Acetylcholine	Ergotamine	Falls increased	Falls decreased	Falls unchanged
Group I								
			mgm./kgm.	*mgm./kgm.*	*mgm./kgm.*			
Dog	4	Nembutal		0.0005–0.005	0.5–2.0		4	
Dog	1	Urethane		0.0025	1.0	1		
Dog	1	Morphine		0.001	1.0	1		
Cat	13	Nembutal		0.000025–0.0005	0.25–1.0	3	10	
Cat	2	Urethane		0.00005–0.00025	2.0	1	1	
Cat	2	Ether		0.0001–0.0005	1.5	1		1
Group II								
Dog	1	Nembutal	5.0	0.1	1.0		1	
Cat	3	Nembutal	0.5	0.005–0.05	1.0–2.0	2		1
Cat	4	Nembutal	3.0	0.005–0.1	1.0	3		1
Cat	1	Nembutal	5.0	0.1	0.25	1		
Group III								
Dog*	1	Nembutal	0.5	0.005	1.0	1		
Dog	1	Nembutal	2.0	0.2	2.0	1		
Cat*	2	Nembutal	3.0	0.005	1.0	2		
Cat	2	Nembutal	3.0	0.3–0.5	1.0	2		

* Animals received 0.5 mgm. of physostigmine in addition to atropine.

occasional animal. In twelve of the fifteen animals of these groups, the vasodilator responses to acetylcholine were augmented by ergotamine.

The effect of additional atropine on the depressor responses to acetylcholine following ergotamine. Table 3 is a summary of the animals in which additional atropine was given following the ergotamine reversal of the acetylcholine pressor effect in an attempt to restore the pressor responses. If acetylcholine after additional atropine produces rises in blood pressure of about the same magnitude as the original rises obtained before the ad-

ministration of ergotamine, then the ergotamine action in producing the reversal is due to sensitization of the vasodilator mechanism to acetylcholine and not to paralysis of the sympathetic endings.

Since there was no apparent difference in the data obtained from cats and dogs, the results in table 3 were not assembled according to species.

TABLE 3

The effect of additional atropine on the ergotamine reversal of the acetylcholine pressor effect

NUMBER OF ANIMALS	ADDITIONAL INTRAVENOUS ATROPINE	BLOOD PRESSURE DECREASE AFTER ADDITIONAL ATROPINE	RELATION BETWEEN THE PRESSOR RESPONSES TO ACETYLCHOLINE AFTER ADDITIONAL ATROPINE AND THOSE (ORIGINAL) OBTAINED BEFORE THE ERGOTAMINE REVERSAL				
			Falls abolished with no rises	Rises about ½ of original	Rises about ¾ of original	Rises about same as original	Falls of 11 to 25 mm. preceding rises the same as or greater than the original
		Group I (animals taken from group I of table 1)					
	mgm./kgm.						
4	5–25	Marked		1	1	1	1
2	30–50‡	Marked			1		1
1	50‡	Slight	1				
		Group II (animals taken from groups II and III of table 1)					
4	5–15	Marked	1†		1†	2	
1	25‡	Marked					1
9	2–5	Slight to moderate		1†	2	4	2*

* One of these animals was given an additional 25 mgm. of atropine intravenously without further affecting the acetylcholine response.

† Barium chloride restored marked rises to acetylcholine in these animals (see fig. 4).

‡ Administered intramuscularly.

In nineteen of the twenty-one animals, pressor responses to acetylcholine were obtained following additional atropine,[1] and more than one-half of

[1] It is often difficult to demonstrate the effect of additional atropine on the depressor effect of acetylcholine following ergotamine. Atropine produced in many cases an abnormally low blood pressure (see table 3) under which condition the acetylcholine pressor effects are usually reduced in size, possibly because the slowed circulation permits a greater destruction of acetylcholine. When good pressor responses to acetylcholine are not restored by 5 to 15 mgm. of additional atropine by vein, it is probable that they will never be restored because of the toxic action of atropine in most animals. Consequently the additional atropine administered was usually limited to this amount, although occasionally as much as 25 mgm. were employed.

these animals showed rises to acetylcholine of about the same magnitude as, or greater than, the original rises to acetylcholine. Figures 1 and 3 show typical tracings obtained in two of the latter animals. The best responses to acetylcholine after additional atropine were usually obtained in animals in which pure initial pressor responses were obtained in the presence of smaller amounts of atropine, and these pressor responses were

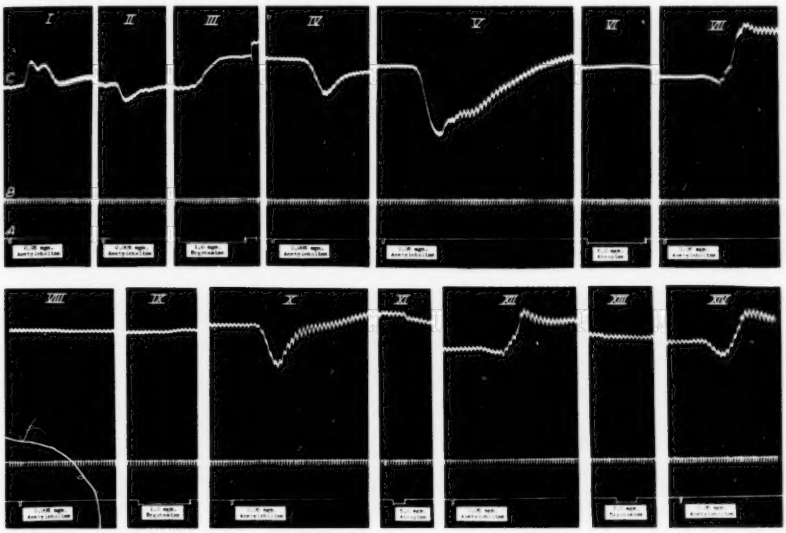

Fig. 3. Showing two reversals of the acetylcholine pressor effect by ergotamine and two restorations of this pressor effect by additional atropine in the same animal; also increased vasodilator responses to smaller amounts of acetylcholine by ergotamine.

A, line indicating injection of drugs intravenously. B, time 1.7 seconds; base line representing zero mm. of Hg pressure. C, blood pressure tracing from the common carotid artery.

Dog, 6.95 kgm., 30 mgm. of Sigmodal sodium, 0.5 mgm. of atropine sulfate and 0.5 mgm. of physostigmine salicylate per kilogram by vein. I. 0.05 mgm. of acetylcholine chloride. II. 0.005 mgm. of acetylcholine chloride. III. 1.0 mgm. of ergotamine tartrate. IV. Same as II. V. Same as I. VI. 5.0 mgm. of atropine sulfate. VII. Same as I. VIII. Same as II. IX. Same as III. X. Same as I. XI. Same as VI. XII. Same as I. XIII. 2.0 mgm. of ergotamine tartrate. XIV. Same as I.

changed into pure depressor effects by no more than 1.0 mgm. of ergotamine. The rises to acetylcholine restored by additional atropine were more delayed than the original pressor responses. This is also true of rises from epinephrine (fig. 1) and of the depressor responses to acetylcholine following ergotamine (figs. 1, 3 and 4).

Figure 3 also shows that after the acetylcholine pressor response is

restored by additional atropine (fig. 3, VII), the administration of more ergotamine again reverses the acetylcholine pressor effect (fig. 3, X), but the fall in blood pressure is less than that which occurred during the first reversal (compare V with X of fig. 3). More atropine again restored

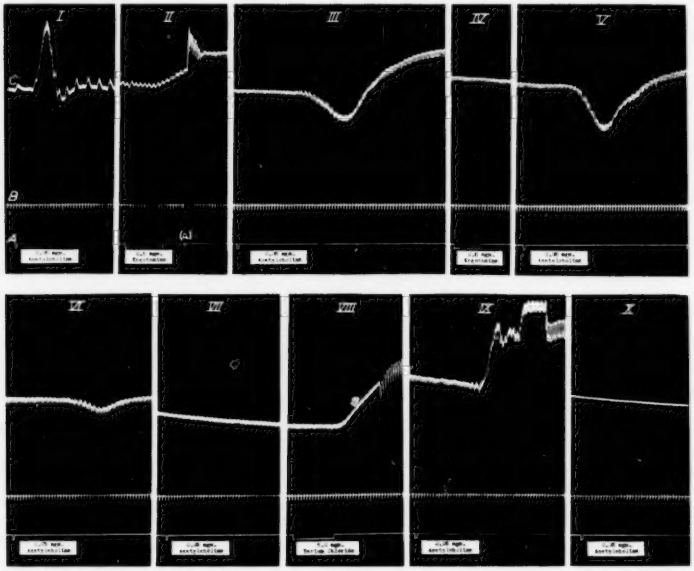

Fig. 4. Showing restoration of pressor response to acetylcholine by barium chloride (additional atropine ineffective) following ergotamine reversal; also effect of more ergotamine on a biphasic acetylcholine response.

A, line indicating injection of drugs intravenously. B, time, 1.7 seconds; base line representing zero mm. of Hg pressure. C, blood pressure tracing from the common carotid artery. (a), drum stopped, tracing continued after 5 minutes.

Dog, 7.2 kgm., adrenalectomized, 30 mgm. of Nembutal, 4.0 mgm. of atropine sulfate and 1.0 mgm. of physostigmine salicylate per kilogram by vein. I. 0.05 mgm. of acetylcholine chloride. II. 0.5 mgm. of ergotamine tartrate. III. Same as I. IV. Same as II. V. Same as I. VI. 0.05 mgm. of acetylcholine chloride following 4.0 mgm. of additional atropine sulfate. VII. 0.05 mgm. of acetylcholine chloride following 4.0 mgm. more of atropine sulfate. VIII. 5.0 mgm. of barium chloride. IX. Same as I. X. 0.05 mgm. of acetylcholine chloride following 4.0 mgm. of nicotine salicylate.

the acetylcholine pressor response (fig. 3, XII). Similar results were obtained in two other dogs and one cat, except that the second reversals of acetylcholine pressor effects by ergotamine were biphasic responses or incomplete reversals (compare with fig. 3, X).

Barium chloride fully restored the pressor responses to acetylcholine

in three animals in which no or small rises from acetylcholine occurred after additional atropine (fig. 4).

In two experiments it was demonstrated that additional physostigmine did not restore pressor responses to acetylcholine reversed by ergotamine as it did in the case of the nicotine reversal previously described (13). The two experiments in which the animals received 5 and 10 mgm. of physostigmine (group IV of table 1) and in which ergotamine reversed the pressor responses to acetylcholine, also indicate that even large amounts of physostigmine had no effect on this reversal.

The effect of ergotamine on the acetylcholine pressor response following large amounts of atropine. If the effect of large amounts of atropine in restoring the acetylcholine pressor effect reversed by ergotamine was due to the complete abolition of the vasodilator action of acetylcholine, then the administration of such large doses of atropine prior to the administration of ergotamine should prevent a reversal of the acetylcholine pressor response by ergotamine. Since these large amounts of atropine increased the toxicity and anesthetic action of barbiturates (17, 21), smaller doses of the anesthetic agent were employed following the administration of atropine (e.g., as little as 10 mgm. of sodium pentobarbital was sufficient to produce anesthesia following 50 mgm. of atropine).

Animals without physostigmine. In two dogs receiving 20 mgm. of atropine each, the pressor responses to 1 mgm. of acetylcholine were reduced about one-half by 1.0 mgm. of ergotamine, while the pressor responses to 0.5 mgm. of acetylcholine were unchanged in one animal but reduced about one-half in the other. In one cat receiving 20 mgm. of atropine, a biphasic blood pressure response was produced by 0.5 mgm. of acetylcholine both before and after 1.0 mgm. of ergotamine, but after ergotamine the falls in blood pressure were greater and the rises smaller. The pressor responses to 0.3 and 0.5 mgm. of acetylcholine in one dog receiving 50 mgm. of atropine were unchanged by the administration of 1.0 mgm. of ergotamine. The blood pressure of these animals remained at a moderate level throughout these experiments.

Animals with physostigmine. In one dog and one cat receiving 25 mgm. of atropine and 1.0 mgm. of physostigmine, 1.0 mgm. of ergotamine rendered the pressor effects from 0.025 mgm. of acetylcholine biphasic. After the administration of 25 mgm. of atropine intramuscularly in addition to the original amount given intravenously, pure pressor responses to acetylcholine were restored. The blood pressure of these animals remained good throughout these experiments although lowered somewhat by the additional atropine.

The pressor responses to acetylcholine in these experiments were unchanged, reduced about one-half or rendered biphasic by ergotamine. The biphasic responses to acetylcholine and perhaps the decrease in the

pressor response may be interpreted as a tendency toward increasing the vasodilator effects of acetylcholine by ergotamine even in the presence of large amounts of atropine.

Discussion. The pressor response to acetylcholine in animals receiving moderate amounts of atropine appears to be the resultant of a marked pressor effect from the liberated adrenergic substances overcoming the vasodilator action of acetylcholine, as is diagrammatically represented in figure 5. Ergotamine produced a reversal of these pressor effects of acetylcholine in intact as well as in adrenalectomized animals. However, when the amount of atropine was large, the pressor responses to acetylcholine were converted by ergotamine into biphasic effects instead of pure vasodepressions.

Since ergotamine reverses the acetylcholine pressor effect, it must act *a*, by inhibiting the liberation of sympathomimetic substances by acetylcholine; *b*, by preventing the chemical mediators liberated by acetylcholine from penetrating into the receptor cells thus permitting the vasodilator action of acetylcholine to become dominant; *c*, by increasing the vasodilator effects of acetylcholine, or *d*, by any combination of these factors.

a. The experiments in which adrenergic substances liberated by acetylcholine were transferred from one animal to another, and those in which additional atropine caused restoration of the pressor effect of acetylcholine reversed by ergotamine, demonstrate rather conclusively that *ergotamine does not interfere with the liberation of sympathomimetic substances.*

It seems plausible to assume that these chemical mediators liberated by acetylcholine in atropinized animals are similar to those produced by faradic stimulation of sympathetic nerves. From the experiments of Cannon and Rosenblueth (4) and Greer and co-workers (6), it is seen that even after as much as 1 to 5 mgm. of ergotoxine the pressor effects from stimulation of the hepatic nerve or of the lower abdominal sympathetic chain were still very marked. Cannon and Bacq (22) report that even when the sympathetic responses are completely abolished by the ergot alkaloids as far as the immediate effector organ is concerned the liberation of the chemical mediators may still be demonstrated by an increase in heart rate. In the experiments presented herein, ergotamine usually did not prevent the cardiac acceleration produced by acetylcholine.

b. The restoration of the pressor response to acetylcholine by additional atropine following the reversal by ergotamine indicates that *ergotamine (0.5 to 2.0 mgm.) does not interfere materially with the penetration of the chemical mediators into the receptor cells.*

As a general rule, cats require more atropine than dogs to obtain pure pressor responses to acetylcholine before ergotamine, and also more atropine to reëstablish pressor responses to acetylcholine which have been reversed by ergotamine. In some animals, a given amount of acetyl-

choline produces a pressor response, whereas a smaller quantity of acetylcholine may cause a fall in blood pressure, the latter dose being too small to affect the sympathetic ganglia.

Since the state of the vasodilator mechanism or the response to various drugs cannot be predesignated, each animal presents an individual problem in which the quantitative relationship between ergotamine, atropine and perhaps acetylcholine must be balanced in a more or less perfect man-

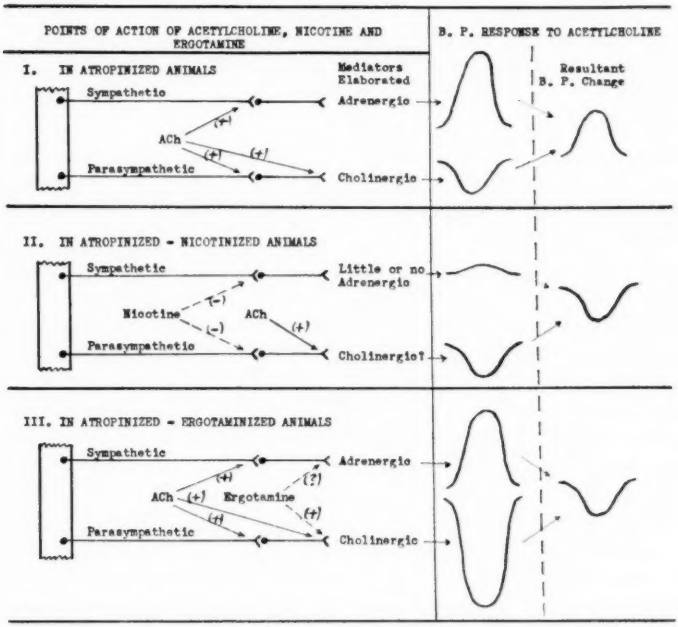

Fig. 5. A tentative schema interpreting the experimental findings.

Points of action of drugs on ganglia or nerve ends indicated by arrows. B. P. = Arterial blood pressure. (+) = Stimulation. (−) = Depression. (?) = Action not determined.

ner in order to obtain pure depressor responses to acetylcholine after ergotamine, or to obtain pure pressor responses to acetylcholine after additional atropine. Certainly this perfect balance existed in the two animals of figures 1 and 3 and in other animals in which similar results were obtained. Since there is no reason at the present time to suppose that atropine can directly oppose any depression of the sympathetic endings by ergotamine, these experiments show that ergotamine does not materially interfere with the penetration of adrenergic substances into

the receptor cells. However, in most cases, the hemodynamic responses to both acetylcholine and epinephrine were delayed following ergotamine.

On the basis of these preceding experiments, the best explanation for the fact that in some animals after additional atropine acetylcholine produced either no or small rises in blood pressure, appears to be that in these animals atropine was either toxic or less effective in overcoming the ergotamine sensitization of the vasodilators to acetylcholine. In other words, the vasodilator responses to acetylcholine practically neutralized the pressor effects of the liberated adrenergic substances in these animals and the resultant blood pressure rises were nil or small. An alternate interpretation, i.e., that ergotamine may depress the sympathetic endings, would also explain these latter experiments, but the bulk of the experimental evidence opposes this view.

c. If ergotamine affects neither the liberation of "sympathins" nor their penetration into the receptor cells, by inference, *ergotamine must sensitize the vasodilator effects of acetylcholine* in reversing the acetylcholine pressor effect. The only disconcerting evidence not supporting this hypothesis is the fact that in non-atropinized animals ergotamine usually decreased the vasodepressions from very small amounts of acetylcholine. Barium chloride and other smooth muscle stimulants were also found to decrease the depressor effects of acetylcholine (23). The action of ergotamine on the vascular system is in many respects similar to that of barium chloride, and apparently this effect of ergotamine, viz., a barium-like action on the contractile elements of the blood vessels or other vascular changes, diminishes the vasodilator action of small amounts of acetylcholine. The increased vasodilator response to acetylcholine after ergotamine in a morphinized animal reported by Rothlin (24) was confirmed.

The hypothesis that ergotamine sensitizes the vasodilator mechanism to acetylcholine is borne out more satisfactorily by the results obtained in atropinized animals. In these animals, the vasodilator effects of much larger amounts of acetylcholine than were employed without atropine were usually augmented by ergotamine. With borderline doses of acetylcholine, i.e., amounts which may produce either vasopressor or vasodepressor effects, the pressor responses were reversed and the depressor responses were increased by ergotamine. Added weight to this hypothesis is offered by the experiments in which ergotamine again reversed the acetylcholine pressor effect restored by additional atropine, and additional atropine again reëstablished pressor responses to acetylcholine (fig. 3).

The ergotamine reversal of the acetylcholine pressor effect differs from that of the nicotine reversal in the following ways: *a*, additional physostigmine does not reëstablish the vasopressor effects of acetylcholine following the ergotamine reversal as it does following the nicotine reversal, and

b, additional atropine in most cases restored good rises in blood pressure from acetylcholine following the ergotamine reversal but not after the nicotine reversal. On the basis of this and other facts presented, the ergotamine and nicotine reversals of the acetylcholine pressor effect must take place by two entirely different mechanisms. It is therefore assumed that *a*, as previously reported (13), nicotine blocks the liberation of chemical mediators apparently by paralyzing the ganglia which are stimulated by acetylcholine, allowing the vasodilator effects of acetylcholine to become dominant, and that *b*, ergotamine does not interfere with the liberation of chemical mediators by acetylcholine or with their penetration into the receptor cells, but rather enhances the vasodilator action of acetylcholine.

SUMMARY

1. Ergotamine decreases or abolishes its own pressor effect upon repeated administration, and causes in most animals cardiac slowing in the presence of atropine. The pressor responses to epinephrine in atropinized animals are not reduced by ergotamine.

2. The pressor responses to acetylcholine in atropinized and in physostigminized-atropinized animals are converted into depressor effects by 0.5 to 2.0 mgm. of ergotamine if the amount of atropine is not too large. This reversal of the acetylcholine pressor effect occurs in intact as well as in adrenalectomized animals.

3. It has been demonstrated that ergotamine does not interfere with the liberation of "sympathin" by the action of acetylcholine on the sympathetic ganglia.

4. Ergotamine decreases the vasodilator responses to acetylcholine in most non-atropinized animals, but increases the vasodilatation from acetylcholine in most atropinized animals, particularly when larger amounts of acetylcholine (approaching those amounts reversed by ergotamine) were employed.

5. Additional atropine restores the pressor responses to acetylcholine (after reversal by ergotamine) in most of the experimental animals. Barium chloride seems to have an additive action with atropine in restoring these pressor responses.

6. It is assumed that, since the amount of ergotamine employed herein does not materially interfere with the responses to agents acting on the sympathetic endings, the action of ergotamine in reversing the acetylcholine pressor effect must be due to sensitization of the vasodilator mechanism.

7. The differences between the ergotamine and the nicotine reversals of the acetylcholine pressor response are discussed.

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CLIMATIC EFFECTS ON THE VOLUME AND COMPOSITION OF BLOOD IN MAN

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It is a matter of common experience that the initial discomfort of extremes of hot or cold weather becomes less after the first few days of exposure. Again, the "comfort zone" of temperature is known to lie between different limits according to the acclimatization of the individual to winter or to summer temperatures (Yaglou, 1926). From this it may be inferred that there must exist slow physiological adaptations which are superimposed upon the rapid mechanisms of adjustment to temperature with which the study of temperature regulation has mainly been concerned. The acute reactions to temperature are evidently slowly modified in their range and in their effectiveness in the course of continuous exposure to extremes of temperature.

Evidence of some of the physiological changes that may underlie such adaptations has already been reported. Griffiths et al. (1929) and Vernon and Warner (1932) found seasonal changes in the circulation. In this laboratory Dr. Ella Roberts (unpublished data, 1930) found that all the capillaries at the root of a nail might be open during hot weather in Philadelphia, so that no further increase could be obtained by the use of histamine; this maximal dilatation, however, was attained only after several days of heat. Bazett et al. (1937) found differences in cardiovascular behavior in hot baths from summer to winter, and Burton and Bazett (1936) noted that constriction in the same subjects in cold baths was more effective in a severe winter than in the summer.

Large alterations in blood volume in response to climatic changes have been reported (Barcroft et al., 1922), though Dill (1938) concludes that these are not concerned in adaptation to hot weather. However, changes in blood volume have been reported from these laboratories which have been apparently related to changes in temperature (Sunderman et al., 1937). The present paper gives these data in more detail.

CONDITIONS OF EXPERIMENTS. *Seasonal variations.* Plasma volumes were measured with the subjects basal in the morning in a room at a com-

fortable temperature. During the winter, periods were chosen which followed several days of cold weather and in the summer, periods following several days of warm or hot weather. The subjects followed their ordinary routine and were exposed to outside temperatures only 1 to 2 hours per day.

Artificially produced variations. The data here reported are derived from four experiments in an air-conditioned room (the air-conditioning unit was kindly supplied by the General Electric Company). The air movement was slight, in the greater part of the room less than 20 ft./min. Variations in room temperature during any experimental period rarely exceeded 0.5 to 1°C. except in the transition periods and at night. Between

TABLE 1

Experiments in an air conditioned room

	CONDITION	DRY BULB	WET BULB	SUBJECTS USED	DURATION
					days
1. Winter (December 26 to January 7, 1937–1938)	Cool	23.6°	14.9°	A and B	1
	Warm	32.5°	20.8°	A and B	5
	Cool	24.6°	16.4°	A and B	1¼
	Warm	32.3°	20.4°	A	4
2. Summer (June 12 to June 24, 1938)	Warm	32.4°	26.3°	A and C	5
	Cool	21.1°	16.0°	A and C	6½
3. Summer (June 6 to June 30, 1939)	Warm	32.1°	26.0°	E and F	4⅓
	Cool	23.7°	18.0°	E and F	6
4. Spring (February 16 to March 10, 1939)	Cool	23.0°	16.2°	C and D	1
	Warm	28.6°	20.6°	C and D	1¼
	Warm	32.6°	24.3°	C and D	5
	Cool	23.7°	17.1°	C and D	2
	Warm	32.4°	23.3°	C and D	4¼

9:40 p.m. and 5:40 a.m. the control was set at a level 0.5 to 1.5°C. lower than during the day. The humidity could not be adequately controlled in the summer. It remained however fairly uniform except in experiment 3. The average conditions during the day are given in table 1. The subjects remained in these conditions throughout each day, except for absences which on any day totalled less than five minutes.

The *routine* of measurements followed each day was kept as far as possible the same in any single experiment. In the warmth the subjects wore little clothing; in the cold they wore as little as was possible without real discomfort. All blood volume measurements were made under morning basal conditions. Measurements of other changes were also made and will be reported later.

METHODS EMPLOYED. *Plasma volume.* Most of the data reported were obtained

by the injection of Congo red. The actual estimates of dye were made on serum. The method of estimation was that described by Sunderman and Austin (1936) somewhat modified.

In experiment 2 the estimates were made by the use of the blue dye T-1824 by the method described by Gregersen (1935). At this time one of us (F. W. S.) was away ill, and the measurements were kindly made for us by Dr. Norman Freeman, to whom we are greatly indebted. In experiment 3 blood volume was estimated from the total hemoglobin by the CO method (see later) and nominal plasma volumes were calculated from these and the hematocrit values.

Hematocrit estimates. A small sample of the blood as drawn was prevented from coagulation by mixing with heparin in a watch glass; it was drawn up into a number of small glass capillary tubes which were sealed in a flame and centrifugalised for 30 minutes to 1 hour. The value utilized was the average of a large number of such tubes. In a few cases heparin was not used but the blood was taken into sodium oxalate according to Van Allen's method; under these conditions two or three samples only were taken. The average values obtained by the two methods were found to agree (e.g., method described 43.6, by Van Allen 43.5; method described 40.6, by Van Allen, 40.2 vols. per cent).

Hemoglobin estimations were made in some experiments. In experiment 1 samples of blood were obtained from the finger, and estimates of hemoglobin content were made as acid-hematin. In experiments 3 and 4 they were made as oxyhemoglobin by a photo-colorimetric technic modified from that described by Hartmann (1937). This method will be described in detail later. For these experiments the method was still somewhat in the experimental stage and only approximately standardized. It was however capable of great accuracy for the estimation of variations from day to day.

Red cell volume and total hemoglobin were estimated in most experiments from the plasma volume, hematocrit and hemoglobin values. The estimates can therefore only be considered as nominal values, since hematocrit estimates of the ratio of the total volumes of erythrocytes and plasma have systematic errors. However in experiment 3 direct estimates of the total hemoglobin were made by the inhalation of carbon monoxide and the utilization of the principles described by Hartmann. As with the hemoglobin values the data so obtained were probably accurate from a relative, but not necessarily from an absolute, point of view.

Plasma or serum protein concentration was measured in experiments 1 and 4 (serum) and in 3 (plasma) by determining the specific gravity by weighing (Moore and Van Slyke, 1930). Where plasma was utilized heparin was used as the anticoagulant. Some estimates were also made (expts. 1 and 2) by the Pregl method.

Colloid osmotic pressure was directly estimated in experiments 3 and 4. In experiment 3 estimates were made in duplicate on each of two blood samples drawn each day. Measurements were made according to the method described by Wells (1933).

Total osmotic pressure measurements were made in experiments 3 and 4 by the vapor pressure method of Roepke and Baldes (1938). In experiment 3 duplicate measurements were made on each of two blood samples drawn each day.

Other measurements. In experiment 1 some estimates of extracellular fluid were made by the injection of sodium thiocyanate at the same time that blood volume was measured. The serum analyses were made according to the technic of Crandall and Anderson (1935). In experiment 1 the serum chloride concentration was measured; the method employed was that described by Sunderman and Williams (1933).

Mouth temperatures were measured with clinical thermometers each morning on waking and usually at night. In subject 1 in experiments 1 and 2 continuous records

of rectal temperature were also made during each night with a Leeds and Northrup recording resistance thermometer.

Subjects used. All the subjects were males. Subjects A and B were those numbered 10 and 8 by Bazett et al. (1935) where they are sufficiently described; they were somewhat older and heavier (surface areas now 1.83 and 1.68 sq. m. respectively). Subject G had recently come from Edinburgh; his age was 29, height 180 cm., weight 70.4 kgm. and surface area 1.88 m^2.

Subjects C, D and E were all Philadelphians. Subject C was 27 years of age, height 183 cm., weight 82.2 kgm. surface area 2.01 m^2. Subject D was 20 years of age, height 180 cm., weight 80.1 kgm. and surface area 1.98 m^2. Subject E was 23 years of age, height 179 cm., weight 86.6 kgm. and surface area 2.04 m^2. Subject F came from Arizona, was 20 years of age, height 182 cm., weight 61.0 kgm. and surface area of 1.79 m^2. Subjects A and C were each utilized twice in room experiments.

TABLE 2

Seasonal variations in blood volume per M^2 body surface

SUBJECT	DATE	CONDITIONS	HEMATOCRIT	PLASMA VOLUME	NOMINAL CELL VOLUME	NOMINAL BLOOD VOLUME
A (age 51–52)	12/22/37	Cool	39.5	1780	1185	2965*
	2/ 8/37	Moderately cold	40.5	2035	1385	3420
	10/30/36	Cool	41.5	2015	1435	3450
	6/24/37	Warm	40.5	2190	1490	3680
	7/13/37	Hot	38.0	2415	1475	3890
B (age 36–37)	12/27/37	Cool	40.8	1660	1145	2805*
	1/14/38	Cool	40.5	1690	1150	2840*
	3/ 5/37	Moderately cold	41.5	1890	1355	3245
	11/ 4/36	Cool	43.5	2110	1425	3735
	6/21/37	Warm	42.5	2255	1670	3925
G (age 29)	3/25/37	Cold	42.0	1565	1135	2700
	11/12/36	Cool	45.2	1915	1585	3500

* Data starred are repeated in table 2, as part of room experiment 1.

RESULTS. *Seasonal variations.* The values obtained for plasma volume and hematocrit as well as the nominal cell and blood volumes calculated from them are given in table 2 for 3 subjects. The data given there were all obtained by the injection of Congo Red, and they are expressed relative to the surface areas of the subject. This forms a convenient method of comparison since many of the other data obtained on these subjects, and to be discussed later (e.g., insensible water loss from the skin) are most readily considered on a surface area basis. It will be noted in the table that the values fall in order according to the prevailing weather conditions with perhaps some additional seasonal factors. The increases in blood volume and cell volume, as well as plasma volume, are so great that possible errors in calculations from hematocrit values cannot be responsible. There is clearly an increase both in the plasma and cell volumes in the summer.

There appears to be no consistent difference between summer and winter in the hematocrit level, except that it is low during the acclimatization to warmer weather, and high during the acclimatization to cooler weather.

Effects produced in an air-conditioned room. Plasma volume, hematocrit, cell volume and blood volume. Data for 6 subjects are presented in table 3 in the same form as those of table 2. It may be noted that the changes produced artificially are entirely comparable to those observed as the result of seasonal changes in climate. The changes in plasma volume produced in a warm room within 5 days are of the same order as those obtained in the summer (see subjects A and B in expt. 1) but the increase in cell volume is much less. The increases in cell volume do not commence for the first two days of heating (expt. 1) and proceed much more slowly, thus causing a fall in the hematocrit value like that observed seasonally. The slow rise prevents the full change in cell volume being attained within 5 days, though it may be noted that both in response to seasonal changes, and in the heated room B made greater changes in cell volume than did A (expt. 1). On cooling also the changes in plasma volume proceed more rapidly that those in the red cells, resulting in a rise in the hematocrit value during acclimatization.

The data given in table 3 were obtained by different methods. The changes observed were qualitatively the same in all except in experiment 4. With this exception there is a consistent slow increase in blood volume in the warmth and decrease in the cold.

In experiment 4, the only experiment conducted in the spring, the blood volume of subject C was not reduced but actually slightly increased when he was examined in the warmth immediately following exposure to cold for 2 days. However, there is evidence that some factors in the process of acclimatization may proceed for some while after the temperature of the environment has been changed. This experiment was conducted to test whether this might be true in relation to blood volume; further experiments are required to decide this point, but the possibility of effects developing with a considerable lag cannot be excluded. Subject D in experiment 4 also showed a similar increase in blood volume following the short exposure to cold but was even more atypical in that he showed a reduction in blood volume in the warmth and also at the end of the experiment when living at the original room temperature. There was a considerable progressive loss of weight, and the circulatory reactions showed atypical changes. This subject had been taking heavy exercise (basket-ball) before being shut up in the small room; the loss of exercise may have introduced complications. Other factors than mere room temperature may play an important rôle.

Though the changes observed with alteration of climate are the same qualitatively, regardless of the method of measurement, the actual values appear to differ. The estimates of plasma volume made by T-1824 seem to be somewhat smaller than those made by Congo Red both in subject A and subject C, if allowance be made for the prevailing climatic conditions.

TABLE 3

Effects of temperature changes in room

EXPERIMENT	SUBJECT	DATE	CONDITIONS AND DURATION	Hb. CONC.	HEMATOCRIT	PLASMA VOLUME	CELL VOLUME	BLOOD VOLUME	SERUM OR PLASMA PROTEIN	COLLOID OSMOTIC PRESS.	TOTAL PLASMA PROTEIN	Hb.
			days	*gm./100 ml.*		*ml. /M²*	*ml. /M²*	*ml. /M²*	*per cent*	*mm. Hg*	*gm. /M²*	*gm./M²*
1	A	12/22/37	C		39.5	1780	1185	2965	6.0		107	
		12/28/37	W 1	15.45	37.5	1975	1180	3155	6.0		118.5	488
		1/ 1/38	W 5	14.8	37.0	2315	1345	3660	6.3		146	542
		1/ 6/38	(C 1½) W 4	14.65	36.5	2290	1310	3600	5.8		133	528
	B	12/27/37	C	15.3	40.7	1660	1145	2805	5.9		98	429
		12/29/37	W 2	13.25	38.0	1870	1145	3015	5.8		108.5	400
		1/ 1/38	W 5	13.7	39.0	2140	1365	3505	6.0		128.5	480
		1/ 6/38	C 5	14.35	41.2	1870	1310	3180	6.4		119.5	456
		1/14/38	C 13		40.5	1690	1150	2840	6.4		108	
2	A	6/13/38	W ½		40.0	1920	1290	3210	6.0		115	
		6/17/38	W 4		38.0	1970	1205	3175	6.55		129	
		6/20/38	C 2		40.4	1720	1160	2880	6.5		112	
		6/24/38	C 6		40.0	1755	1175	2930	6.8		119.5	
	C	6/ 8/38	W		42.0	1665	1200	2865				
		6/13/38	W ½		41.8	1810	1295	3105	6.2		112.5	
		6/17/38	W 4½		42.5	1782	1323	3105	6.55		116.5	
		6/20/38	C 2½		42.9	1750	1310	3060				
		6/24/38	C 6½		42.0	1676	1219	2895	6.7		112.5	
3	E	6/23/39	W 4½	14.85	41.0	1400	970	2370	6.35	24.1	89	360
		6/24/39	C ¼	15.65	43.1	1180	895	2075	6.7	24.7	79	325
		6/25/39	C 1¼	16.1	43.0	1035	790	1825	6.6	26.4	68	297
		6/26/39	C 2¼	15.7	42.5	1090	805	1895	6.4	26.9	70	298
		6/27/39	C 3¼	15.95	43.6	1070	825	1895	6.4	24.5	69	298
		6/28/39	C 4¼	15.55	43.8	1230	955	2185	6.6	25.3	81	340
		6/29/39	C 5¼	16.1	43.4	1165	890	2055	6.4	24.7	75	332
		6/30/39	C 6¼	15.5	41.5	1260	890	2150	6.4	25.0	81	334
	F	6/23/39	W 4½	14.45	38.7	1795	1135	2930	7.2	26.1	129	425
		6/24/39	C ¼	15.1	41.2	1600	1120	2720	7.6	27.5	122	412
		6/25/39	C 1¼	15.25	42.2	1145	835	1980	7.7	30.6	88	302
		6/26/39	C 2¼	15.2	42.4	1215	895	2110	8.2	29.2	100	320
		6/27/39	C 3¼	15.3	43.1					30.0		
		6/28/39	C 4¼	15.45	42.7	1235	920	2155	8.1	30.3	100	333
		6/29/39	C 5¼	15.4	42.7	1425	1060	2485	7.85	30.7	112	383
		6/30/39	C 6¼	15.85	44.1	1175	925	2100	8.4	31.0	99	332

TABLE 3—*Concluded*

EXPERIMENT	SUBJECT	DATE	CONDITIONS AND DURATION	Hb. CONC.	HEMATOCRIT	PLASMA VOLUME	CELL VOLUME	BLOOD VOLUME	SERUM OR PLASMA PROTEIN	COLLOID OSMOTIC PRESS.	TOTAL PLASMA PROTEIN	Hb.
			days	*gm./100 ml.*		*ml. /M²*	*ml. /M²*	*ml. /M²*	*per cent*	*mm. Hg*	*gm. /M²*	*gm./M²*
4	C	2/17/39	C (prelim.)	(15.9)*	43.5	1785	1370	3155	6.35	(23.3)*		(502)
		3/ 3/39	W 5	14.15	39.5	2135	1395	3530		19.6		500
		3/ 6/39	(C 2) W ¼	15.55	41.3	2155	1510	3665	6.25	22.6		570
		3/10/39	(C 2) W 6	14.05	38.6	2005	1270	3275	6.1	22.1		460
	D	2/17/39	C (prelim.)	(14.1)*	40.4	2060	1400	3460	6.7	(21.0)*		(487)
		3/ 3/39	W 5	14.65	40.1	1965	1335	3300		23.5		484
		3/ 6/39	(C 2) W ¼	14.4	39.1	2250	1455	3705	6.75	27.2		534
		3/10/39	(C 2) W 6	14.45	39.1	1900	1215	3115	6.3	25.0		450

C indicates cool and W warm conditions.

In experiments 1 and 4 plasma volume was estimated by Congo red, in experiment 2 by T-1824. In experiment 3 blood volume was estimated from hemoglobin concentration and total hemoglobin following inhalation of CO. Colloid osmotic pressure was read directly. Serum protein concentration was estimated by specific gravity in experiments 1 and 4 and plasma protein similarly in experiment 3. Serum protein was determined by Pregl in experiment 2. The other data are derived and nominal values. The starred values of experiment 4 represent those observed immediately preceding the warming and not on the preliminary day when blood volume was measured. The last set of values for subject A in experiment 1 and the last two sets of values for both subjects in experiment 4 represent observations in warmth following a period of interruption by cold.

The blood volumes calculated by the use of carbon monoxide also are lower[1] than those obtained under comparable conditions with dyes; this agrees with much of the older literature. Simultaneous comparisons of the various methods are required and have been attempted. It is hoped that it may be possible to report adequate comparisons at a later date The data obtained by carbon monoxide (which could be repeated daily) indicate a rapid reduction of blood volume on cooling with later phasic variations. The chance sampling at irregular intervals employed in the dye methods may miss the greatest change.

Changes in red cell count, hemoglobin concentration and total hemoglobin. The greater rapidity of the changes in the volume of the plasma than in that of erythrocytes causes fluctuations also in the red cell counts and in the hemoglobin concentration. The changes that were observed in

[1] This discrepancy is less if the constants used by Hartmann are employed. The values given are those obtained by the use of constants determined from estimates of O_2 capacity by Van Slyke's method.

experiment 1 are indicated in figure 1. The calculations of the total circulating hemoglobin given in table 3 are admittedly liable to considerable error, but the increase in total hemoglobin on warming so estimated for experiment 1 is entirely supported by the opposite change on cooling observed in experiment 3 and measured more directly. There was also in subject A in experiment 1 an increase in reticulocytes from 0.1 per cent on December 28 to 0.3 per cent on January 1 and 0.4 per cent on January 6.

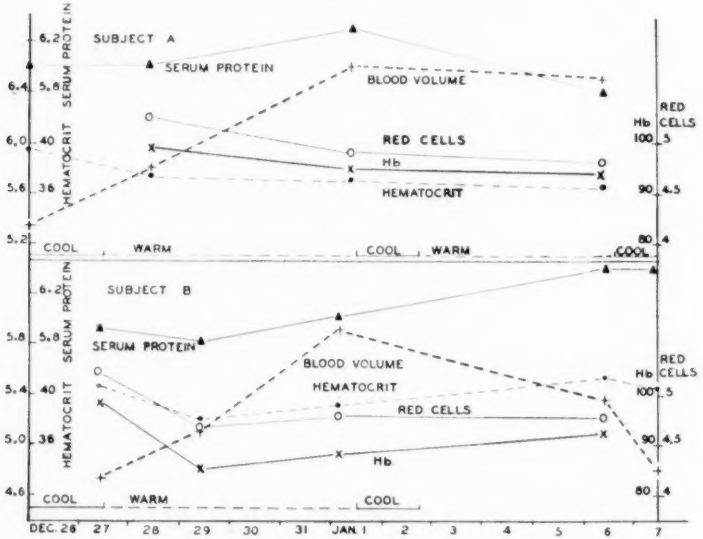

Fig. 1. Data obtained on subjects A (above) and B (below) in experiment 1. The dates of observation are shown as abscissae, and as ordinates serum protein concentration in grams/100 ml., hemoglobin concentration, red cell count in millions/c. mm., hematocrit estimates of the percentage volume of the erythrocytes, as well as the nominal blood volumes calculated from plasma volumes and the hematocrit values. Scales to the left indicate blood volume in liters, serum protein concentration and hematocrit and to the right the red cell count and hemoglobin percentage (15.6 grams per 100 ml.) (considered 100 per cent).

No such increase was seen in subject B, but rather a decrease; examinations have not been made in the other subjects. In view of the invariable increase in the estimates of the total circulating hemoglobin with warmth, and the marked increase in reticulocytes reported in such conditions by Barcroft et al. (1922), stimulation of new formation of cells can be assumed.

In some of the other experiments daily estimates of hemoglobin concentration and hematocrit values were made. These daily observations are indicated in table 2 for experiment 3 where small quantities (100 ml.)

of CO were also inhaled daily. The values of experiment 1 and experiment 3 indicate that a marked change in hematocrit is normally accompanied by a considerable change in blood volume. The changes in plasma volume, and hematocrit and in the blood volume calculated therefrom in experiment 2 are shown in figure 2. The changes in the hematocrit on June 19 in both subjects indicate the presence of considerable changes, and it

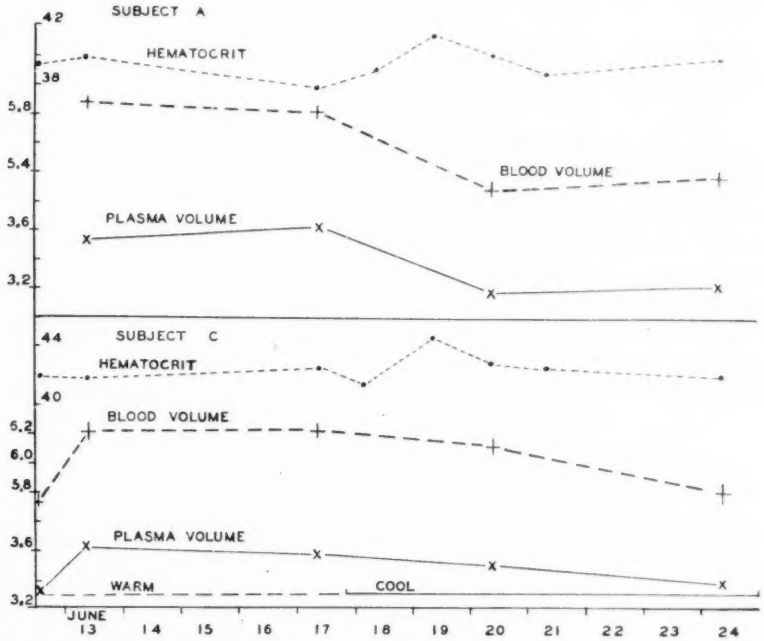

Fig. 2. Data obtained on subjects A (above) and C (below) in experiment 2. The dates of observation are shown as abscissae, hematocrit estimates of the percentage volume of erythrocytes, plasma volumes and the nominal blood volumes calculated therefrom as ordinates. The scales indicate hematocrit percentage values, and the volumes of the blood and plasma in liters.

is likely that the estimates of plasma volume made a day later failed to indicate the rapidity and magnitude of the initial changes in blood volume.

Changes in plasma protein concentration, colloid osmotic pressure and the total circulating plasma protein. There may be some initial reduction of the plasma protein concentration when a subject adapted to cold is suddenly exposed to heat (expt. 1, subject B). The single observation on this subject was confirmed in experiment 3 on subject F (fig. 3). On the other hand no indication of any initial fall was detected in experiment 1

on subject A nor in experiment 3 on subject E (fig. 3). The increased volume of plasma seems to be associated normally with an increase in the total plasma protein in circulation, indeed the latter increase is usually

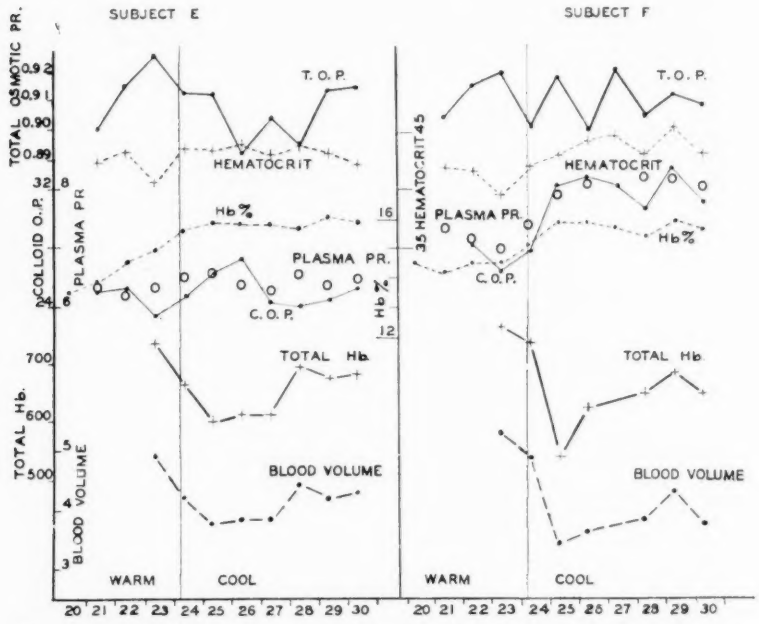

Fig. 3. Data obtained on subjects E (to the left) and F (to the right) in experiment 3. The dates of observation are shown as abscissae and as ordinates values for total osmotic pressure of the plasma, percentage of red cells by hematocrit, hemoglobin concentration in grams/100 ml., plasma protein concentration in grams/100 ml., colloid osmotic pressure of the plasma in mm. Hg, total circulating hemoglobin in grams, and estimated blood volume in liters. Scales for total osmotic pressure, colloid osmotic pressure and total hemoglobin are set to the extreme left; values for total osmotic pressure (T. O. P.) are expressed in terms of percentage concentration of a comparable solution of NaCl. Scales for plasma protein concentration and blood volume are also set to the left. Scales for the hematocrit values and for hemoglobin concentrations are set in the center. The observed values of colloid osmotic pressure labelled C. O. P. are shown by dots connected by a continuous fine line. The protein concentrations estimated for the same blood samples by measurements of specific gravity are shown as open circles unconnected by any line.

the greater so that at the end of a period of prolonged warmth the protein concentration is usually higher. On the return of the subject to cool conditions there is a further rise in the protein concentration, which may be short-lived as in experiment 3 subject E, or of considerable duration as in

experiment 3 subject F, and in experiment 1 subject B. These general changes are observed whatever the method used for estimation. The excellent agreement between the general stability of the protein concentration and that of the colloid osmotic pressure in subject E in experiment 3 both on warming and cooling and the relative instability demonstrated by both methods of measurement in subject F in the same experiment indicate that the methods utilized were adequate to demonstrate the general character of the changes.

The consistency of the data is good but not absolute as may be seen in table 3. Some of the variations observed are dependent on individual differences as for instance those in plasma protein concentration in subjects E and F just referred to. On the other hand the most remarkable character of the experiments was the relative absence of such individual differences between two subjects kept together in the room in any single experiment. The main differences are seen between experiments rather than between subjects. Apparently the control of the experimental conditions either in the room or in the previous history of the subjects was inadequate. Even in the aberrant results of experiment 4 there was some agreement between the two subjects as is indicated in table 3. The marked similarity between the reactions of two subjects is well shown in experiment 3, which will be referred to also in a later paper in regard to cardiovascular changes. The data of this experiment are shown therefore also in figure 3 in a somewhat different form.

Two blood samples were taken with an interval of 20 to 25 minutes, one before and one at the end of the inhalation of 100 ml. of carbon monoxide in oxygen. During this short period changes in the blood occurred, usually small in subject E and often large in subject F. Thus the standard deviation of the variation in hemoglobin percentage between the two samples was in E $\pm$ 0.30 gram per cent and in F $\pm$ 0.69 gram per cent. The actual change in F might be as much as 8 per cent and appeared to be associated with discomfort in breathing into a spirometer. Measurements of hematocrit, hemoglobin per cent, plasma protein per cent, colloid osmotic pressure and total osmotic pressure were made on both samples. The changes in all 5 independent measurements were commonly in the same direction, so that real physiological changes rather than experimental errors in measurement must have been concerned. Thus in subject E the changes were on the average in the same direction in 68 per cent of these 5 measurements, and in F in 82 per cent. For example on June 25 in F the changes were: hematocrit 42 to 44 per cent of erythrocytes, hemoglobin 13.06 to 14.07 grams per 100 ml., plasma protein 7.7 to 8.2 grams per 100 ml., colloid osmotic pressure 30.2 to 37.6 mm. Hg and a total osmotic pressure from one equivalent to 0.914 per cent NaCl to one of 0.925 per cent. In consequence of the large changes in a short period in F, it was difficult to be sure of the fundamental changes from day to day. Consequently both for E and F the values given in table 3 are those calculated from the first blood sample with the subject comfortable; the values shown in figure 3 are those calculated from the means of the two samples. It may be noted that the main character of the changes is the same by both methods of analysis.

Throughout the blood examinations fairly consistent variations were found in the volume color index (ratio of hemoglobin concentration to cell volume). This was usually (expt. 3) in the direction of a relatively increased cell volume in the earlier days of exposure to cold. No deductions can however be drawn as to the normal size of the red cells for under such conditions the venous blood is highly unsaturated with oxygen and mere venosity should theoretically increase the size of the red cells.

Experiments were made by one of us (J. D.) to determine whether the effect of venosity on cell size was measurable by the methods employed. They demonstrated that it was measurable not only in vivo by comparison of bloods drawn from limbs of the same subject kept at different temperatures but also in vitro as the result of equilibrating blood with different gas mixtures. These data will be presented separately.

Total osmotic pressure. Data on experiment 3 are presented in figure 3. There is some evidence of a phasic variation in subject E but in F fluctuations were irregular. The available data indicate a possible slight increase during acclimatization to warmth and inconsistent changes on later exposure to cold. Total osmotic pressure usually varied (somewhat surprisingly) with other factors in the changes seen in F between the two blood samples.

Serum chloride concentration. Estimates were made in both subjects in experiment 1. In the cool the initial concentrations were 102.5 and 103.0 m.Eq./liter in the two subjects; they rose on the 1st and 2nd day of warming respectively to 104.6 in A and 106.1 in B; fell to 102.8 in A and 104.4 in B on the 5th day of warming. In A following a second period of warmth for 4 days the Cl concentration remained on January 6 at 102.6; in B on the same date after exposure to cool conditions for 5 days it was at the original level (103.1) and had fallen to 102.4 8 days later. The changes were slight but similar in direction to those indicated, though indefinitely, in total osmotic pressure in experiment 3.

Extracellular fluid. Observations were made by sodium thiocyanate only in experiment 1. Subject A had body water of 16.0 liters amounting to 23.5 per cent of his weight on December 22 on a preliminary cool day when his plasma volume was 3.23 liters; it amounted to 14.95 liters or 21.6 per cent of his body weight on January 1 after 5 days of heat; his plasma volume was then 4.15 liters. In the same experiment, subject B had an estimated body water of 13.0 liters or 23.6 per cent of his body weight on January 1 at the end of the period of warmth; his plasma volume was then 3.595 liters. After 13 days' exposure to cool conditions the body water was 9.7 liters or 17.7 per cent of his weight, when his plasma volume was 2.84 liters. The changes in the two subjects appear inconsistent with each other.

The changes in *body temperature* and in *weight* will be considered in

more detail in later papers dealing with other aspects. It need only be stated here that no great change in deep body temperature resulted; usually there was in the warmth an initial increase of about 0.5°C. which was reduced to about 0.25° as acclimatization developed. The increase in blood volume developing in the warmth might be associated with a marked increase in weight. This was particularly in evidence in experiment 1. In later experiments it became obvious that such changes in fluid balance might be counteracted by others having the opposite effect, so that the changes in body weight were inconsistent.

Discussion. The existence of considerable changes in blood volume cannot be denied. Increases develop both in plasma volume[2] and in the volume of red corpuscles in the warmth and decreases in the cold, and both changes occur more rapidly in the plasma than in the cells. The magnitude of these changes is considerable but measurements made by different methods appear discordant with one another as far as the absolute values are concerned. It would appear however that blood volumes increase when there is a demand for an enlarged vascular bed to attain vasodilatation in the skin. The enlarged blood volume is associated at first with lowered hemoglobin and cell concentrations but the plasma protein is only lowered very temporarily and rapidly regains a normal or even a raised value. On cooling both the hemoglobin and plasma protein concentrations are raised. The raised levels may both be maintained for some days. However in some subjects the normal plasma protein concentration appears to be rapidly regained. Whether estimated directly by carbon monoxide inhalation or indirectly from plasma volume measurements, there appears to be a rapid loss of hemoglobin from the circulation when the subject is cooled. An increase in hemoglobin produced by experimental heating for 5 days seemed to have disappeared entirely in two weeks (expt. 1, subject B). On the other hand the daily study conducted in experiment 3 seems to indicate that sudden fluctuations may occur during this period of reduction in plasma and red cell volumes In both subjects large amounts of hemoglobin seem to disappear precipitately and later to reappear in lessened amount. While an increased rate of destruction of hemoglobin may have been in existence, such rapid changes can more readily be explained as the result of temporary removal of hemoglobin from the circulation with storage in some reservoir where it is not available for combination with carbon monoxide. According to Hanak and Harkavy (1924) such conditions are found in the spleen.

Evidence will be presented in later papers that warmth, if it be extreme, may give symptoms of dehydration with cardio-vascular inefficiency.

[2] More recent experiments in a warm room for periods of longer duration give some indication that increases in blood volume produced by warmth may be somewhat less marked at a later stage of acclimatization.

Presumably under such conditions there is a reduction, absolute or relative, in blood volume. Daily studies of blood volume have not yet progressed to the experimental study of this stage. The possible relation of the changes in blood volume to those in the circulation and their function from the point of view of heat exchange will be discussed in later papers.

CONCLUSIONS

Exposure of an individual to environmental temperatures maintained at different levels for periods of several days is accompanied by significant changes in blood volume. These changes are found whether blood volume be measured by injection of congo red or T-1824 into the plasma or by the combination of carbon monoxide with hemoglobin. Blood volume is increased in the warmth, decreased in the cold. The changes are associated with changes in the same direction both in the total circulating hemoglobin and in the total plasma protein so that at acclimatization there is little change in their concentrations. The changes in plasma volume develop more rapidly than those in the cells and the initial changes in blood volume are consequently associated with temporary changes in the opposite direction in hemoglobin concentration and hematocrit and sometimes also in the plasma protein concentrations.

We would like to express our thanks to the John and Mary R. Markle Foundation and to the Faculty Research Fund of the University of Pennsylvania for financial support, and to the General Electric Company for providing an air-conditioner. We would also like to thank Dr. Norman Freeman for his generous help in conducting all the blood volume estimates of experiment 2, to Dr. A. J. Creskoff for red cell counts and other blood examinations, to Miss M. E. Maxfield for assistance in measurements of blood volume and to Mr. L. Godfrey and others not only for assistance but also for acting as subjects.

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SLOW ADAPTATIONS IN THE HEAT EXCHANGES OF MAN TO CHANGED CLIMATIC CONDITIONS

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Previous papers from this laboratory have demonstrated that in the course of several days of exposure to changed climatic conditions, whether natural or artificial, there is a considerable alteration in the circulating blood volume (see Bazett et al., 1940). In view of the increased physiological comfort that ensues, it seems likely that the blood volume change is associated with slow adaptations in the heat exchanges of the human body with its environment, with respect to both the total heat loss and its partition into losses by radiation, convection and evaporation. Presumably a partition is eventually achieved in the new environmental conditions which imposes least strain upon the organism. It was therefore of interest to study the data obtained in acclimatization experiments with regard to the heat exchanges.

Details of the environmental temperatures and humidities in four such experiments have already been given in the paper on the blood volume changes (Bazett et al., 1940). In each experiment there were two subjects (six different subjects in all). One experiment was made in winter, two in summer and one in spring. The subjects remained for several days in an air-conditioned room, the temperature of which was adjusted to give several days of warm followed by more days of cool conditions, or the reverse. The sequence in a given experiment will be plain from the tables included in this paper.

Data from which the heat exchanges were deduced consisted of oral temperatures at frequent intervals; continuous records of rectal temperature through the night in two experiments on one subject; weights night and morning and also before and after control periods to the nearest gram by a Tromner balance; complete records of food and water ingestion and of urine and feces excretion; and determinations of skin temperature. Contributory evidence was supplied by the measurements on the circulation to be described in another paper.

RESULTS. *Deep body temperature.* When a period of heat followed cold, there was a slight rise, at most amounting to 0.5°C., in the rectal and oral temperatures at corresponding times in the diurnal cycle. Similarly a slight fall was seen when cold followed warmth. These shifts

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decreased as adaptation to the new conditions proceeded. The heat regulating mechanism was therefore adequate to maintain a thermal steady state within the normal range of body temperature in all of the experimental climatic conditions. The slight shift in the steady state value corresponds to that seen in most physical thermoregulating devices when the thermal demand of the environment is changed. The average temperature of all the body tissues, however, changed considerably in the different environmental conditions.

Caloric intake. Estimates of total heat production, and therefore of total heat loss in the steady state, are often made from the caloric intake in the foods. Complete computations of the caloric value of the food taken each day were made in all experiments, and in two experiments of

TABLE 1

Average caloric intake per day

EXP.	SEASON	SUBJECT	WARM		COOL		PER CENT INCREASE
			Days	Cals./ Sq.M/day	Days	Cals./ Sq.M/day	
1	Winter	A	4	1294	1	1380	7
		B	4	1481	1	1536	3
2	Summer	A	4	1120	6	1344	20
		C	4	1270	6	1602	26
3	Summer	E	4	1037	6	1097	6
		F	4	1097	6	1402	28
4	Spring	C	10	1304	4	1419	9
		D	10	1391	4	1435	3

protein, fat, carbohydrate and water contents. The tables of Bulletin no. 286 of the Connecticut Agricultural Experimental Station (1937) were used.

Daily caloric intake in any period of constant temperature fluctuated considerably. In the hot room, there was often a day on which the subjects suffered headache and loss of weight, and here the caloric intake might be as much as 20 per cent below the average for the period. In the cool conditions fluctuation was less; the mean variation for all experiments was 10 per cent. In view of this it is certainly not justifiable to assume that heat production in any 24 hours balanced the caloric intake. Storage of foodstuff in the body or burning of body tissues must have occurred, with restoration of the metabolic balance in subsequent days.

When the average caloric intake per day is calculated for a period of constant temperature (table 1), there seems to be a significant increase

in the cold periods over the adjacent warm periods. This was most marked in the summer experiments. The appetite is considerably increased in cool conditions and this increase is immediate. It is interesting to note that in the case of subject A, who was common to a winter and a summer experiment, that the caloric intake was 16 per cent greater in the hot room in winter than in summer. The persistence of a "winter appetite" may be indicated.

Detailed analysis in experiment 2 showed that there was a shift in the choice of food from carbohydrates to fat when the temperature of the room was lowered. The equivalent R.Q. of the food was 0.84 $\pm$0.008 for A and 0.86 $\pm$0.002 for C in the hot room, falling to 0.82 $\pm$0.01 and 0.83 $\pm$ 0.001 respectively in the cold. Foods of greater caloric value per gram were chosen.

Evidence will be given later than the increase in caloric intake in cool conditions cannot be taken to indicate an increased heat production. Indeed basal and non-basal metabolism was temporarily subnormal. During the process of acclimatization daily metabolic balance may be far from achievement. Increased appetite, however, undoubtedly is one feature of the physiological process of acclimatization to cold.

Evaporative loss. One item of the heat exchanges may be calculated with considerably accuracy, namely, the heat loss by evaporation. Newburgh and his associates (1937) have developed the method of estimation of evaporation from the body by weighing and have shown that it possesses considerable reliability. By subtraction of the total output of weight in a 24 hour period, consisting of losses by urine, feces and "oxidative loss," from the total intake in the same period by food and water, the expected change in weight may be calculated. Comparison of this with the actual change of weight in the period gives the missing item of weight loss, namely, that by evaporation of sensible and insensible perspiration. The only item of the calculation subject of uncertain error is the "oxidative loss." For its estimation the standard method was employed which assumes an R.Q. of 0.83. In the second experiment a more accurate calculation was also made from the actual weight of protein, fat, and carbohydrate taken, but the results differed insignificantly.

The estimate of "oxidative loss" rests upon the assumption that there is metabolic balance between the food taken in and that oxidized in a 24 hour period. This assumption is certainly not accurately true here, but since the total oxidative loss for any day averages only 100 grams, the error must be relatively small.

In the hot periods with a high humidity where sweating was copious, there may have been some loss of weight by the dropping of unevaporated sweat from the body, so that all the weight loss would not be "evaporative loss." Gagge et al. (1938) have shown that the eventual evaporation of moisture trapped in the clothing has the same end result in the removal of heat from the subject as if it had been evaporated directly from the skin. The loss of weight by unevaporated sweat is in all probability a negligible item in these experiments.

The loss of weight by evaporation was calculated for each 24 hours of the experiments. Since weights were recorded in the evening as well as in the morning, night losses were very simply calculable. Subtraction from the 24 hour losses gave the amounts evaporated in the daytime. The results of the calculations for 24 hour losses are shown in table 2. In the hot rooms the day losses averaged some 65 per cent greater than the night losses, as would be expected when sweating, which depends so much upon activity, was a major factor. In the cold periods the difference was on the average 35 per cent. Since the amount of sweating must have depended greatly upon the activity of a particular day, the night losses are a more reliable guide to adaptive trends than the day

TABLE 2

Evaporative loss for 24 hour periods, in gm./Sq.M/hr.

			COOL		WARM																		
1	Winter	A	42.7	63.5	66.7	64.7	71.3	52.7															
		B	34.5	56.3	76.6	57.7	67.8	42.2															
			WARM								COOL												
2	Summer	A	65.0	82.1	67.6	72.3	57.6	21.7	9.8	16.0	20.0	23.4	25.8										
		C	76.1	70.8	69.2	64.8	63.0	22.3	23.2	17.2	22.4	25.2	20.8										
			WARM						COOL														
3	Summer	E	71.6	60.6	61.9	67.8	24.1	16.3	11.6	9.4	16.1	19.5											
		F	64.8	42.5	47.5	56.9	21.4	17.9	16.4	24.6	22.4	21.0											
			COOL				WARM								COOL				WARM				
4	Spring	C	30.1	27.9	43.4	68.6	58.6	64.2	61.8	57.0	24.9	17.4	64.8	63.2	54.8	84.2							
		D	29.2	18.3	50.9	68.3	58.0	57.7	63.2	50.3	14.1	10.2	21.3	60.2	60.6	61.0	*						

* The response of this subject was atypical in many respects.

losses in the hot periods. In the winter experiment (1) there was for each subject an initial period of increasing loss by evaporation, which was followed in the later days by a decrease. A short period of warm weather preceded the summer experiment (2), in which some of the initial phases of adaptation may have already developed. Consistent with this assumption is the fact that in the summer experiment the initial rise in evaporation was absent for subject C, and lasted only one day for subject A. The subsequent progressive decrease was well marked for both subjects, and was shown by both day and night losses. In experiment 3, the hot period was of four days' duration only and was complicated by a drop of room temperature on the third day (due to a failure of the electrical supply circuit) so that no conclusion may be drawn. In the first hot period of experiment 4 again an initial rise in evaporative loss followed by a decrease

is indicated. Conclusions from the second period of warmth cannot be drawn for this was short and preceded by only two days of cold. From the whole group of experiments the tentative conclusion may be drawn that in acclimatization to conditions of high temperature the loss of heat by evaporation first rises and then progressively falls so that after full acclimatization it is not so great a fraction of the total heat loss (table 3). In this respect, however, as in the case of the circulatory changes, the results appear to depend a great deal upon the physical activity of the subject and upon the severity of the environmental heat.

In the cool conditions the results were more consistent, as might be expected where sweating is absent. Following a lowering of the room

TABLE 3

Heat loss by evaporation for 24 hour periods, as percentage of caloric intake

			COOL		WARM												
1	Winter	A	43	60	92	74	68	56									
		B	31	54	80	64	67	59									
			WARM						COOL								
2	Summer	A	92	93	86	88	65	22	11	16	19	25	27				
		C	86	(103)	86	(109)	89	25	23	17	29	24	24				
			WARM					COOL									
3	Summer	E	74	85	57*	(104)	42	28	15	10	19	24					
		F	90	54	50*	73	19	24	15	25	22	19					
			COOL		WARM						COOL		WARM				
4	Spring	C	30	29	45	72	61	67	64	59	25	18	68	66	57	88	
		D	29	18	54	72	61	61	66	53	14	10	23	64	64	64	

* The temperature of the room was accidentally lower during the night of this period.

temperature there was a gradual fall of evaporative loss from a value which might be above normal in the first 12 hours to a very low minimum value, reached in experiments 2 and 3 on the 2nd, 3rd or 4th day of cold (table 2, also fig. 1). These low values were again seen on the second of the two days of cold of experiment 4 (table 2 and fig. 2). The values reached are strikingly below any which are accepted as standard for these environmental conditions. Gagge (1937) and Gagge, Winslow and Herrington (1938) found, for a number of subjects, that when the temperature is below 25°C. the evaporative loss reaches a minimal level of about 14 cals./sq.M/hour (24 gm./sq.M/hour) when about 25 per cent of the total heat loss is carried by evaporation. Table 3 (where evaporative heat loss is expressed in a percentage of the caloric intake rather than

total heat loss which was not known) shows some minimal values much below this figure. At the end of the cold periods, the evaporative loss had risen until it was in the range of accepted standards.

The abnormality of these low values of evaporative loss was so striking that it was desirable to have measurements made under controlled conditions, so that it would be possible to determine whether physiological or merely physical conditions were responsible. Accordingly in experiment 3, weight losses were determined each morning with the subjects basal during two experimental periods, one of an hour, another of two hours' duration in which the subjects lay nude on the bed. At the beginning and end of each of these periods, skin temperatures, room temperatures and humidity were measured. Basal oxygen consumption was also determined by a

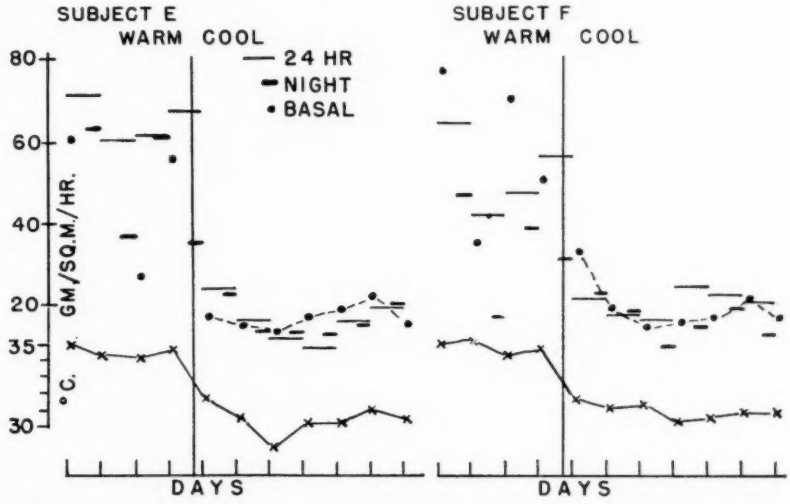

Fig. 1. Weight losses due to evaporation and average surface temperatures (below) of the two subjects in experiment 3.

Sanborn machine. Figure 1 shows some of these results. In the hot room the three values plotted, the 24 hour evaporative loss, night loss and loss in the two hour basal period, are widely scattered. In the cold, however, all agree in showing the fall in the course of three days to subnormal values.

Skin temperatures. These were taken by means of a thermocouple similar to that described by Bazett and McGlone (1927) of uncovered light wire. Fourteen different points on the anterior body surface were used, corresponding to numbers 26, 52, 53, 55, 11, 11a, 18, 18a, 2, 3, 1, 4, 7 and 8 of the points described by Benedict (1925), together with the palmar surfaces of the 3rd fingers. From the mean of temperatures before and after the experimental periods an average temperature for the whole surface was calculated.

Changes in the average skin temperatures are plotted in figure 1. They fell progressively, in each subject, for the first three or four days of cold

to a minimum closely related in time to the evaporative minima. The slow changes were closely correlated with corresponding changes found in the blood flow of the fingers (to be described later). The curve of basal evaporative loss was so closely parallel to that of skin temperature (fig. 1) that a causal relationship was suggested. Whether the low value reached by skin temperature was sufficient to explain the subnormal minima of evaporative loss may be decided by the method of Gagge (1937).

"Wetted area" of the body surface. The evaporation of moisture per square meter of body surface is proportional to the gradient of vapor pressure between the skin and the surrounding air. This is calculated as the difference between saturated vapor pressure at the temperature of the skin and the partial vapor pressure of the air (given by air temperature and relative humidity). If the evaporative loss actually occurring is divided by this pressure difference, a figure is obtained which depends (for a given air movement) only upon the physiological condition of the skin, not upon the purely physical factors. Gagge (1937) finds that this quantity ($W\mu$) varies between a maximum of 28.5 calories per sq. M. per hour per cm. Hg pressure difference and a minimum of about 2.9. If the maximum represents a body surface which, due to sweating, is completely wet, normal insensible loss without sweating represents a percentage wetted area of 10 per cent. The results so expressed for the basal periods of experiment 3 are given in table 4. The values of the quantity ($W\mu$) are in close agreement with those found by Gagge. The minimum values and the corresponding wetted area do not fall below his. The very low values of evaporative loss found in the basal periods in the cold room are then entirely explicable as due to the low values of skin temperature reached in this stage of acclimatization.

However, the low values of the percentage 24 hour evaporative loss remain to be explained. The caloric intake, to which the percentage heat loss by evaporation is referred in table 3, might on these days of the cold be greatly in excess of the heat production. However, even if the metabolism remained throughout the day at the basal value without any specific dynamic action of the food, the percentage loss of evaporation would still fall below normal limits in many cases. There must therefore be some disturbance of the normal conditions of filtration of water through the skin to be evaporated as insensible perspiration so that the average wetted area throughout the day remains less than the normal 10 per cent.

Filtration of water to the skin. If large changes of body fluid and of blood volume are occurring in adaptation to climatic temperatures, a period may well exist where changes in blood protein and cells have not kept pace with the volume changes. Blood concentration with an increased colloid osmotic pressure may result in the initial stages of cold adaptation. We are accustomed to speak of an "equilibrium" between

the mean capillary pressure and the colloid osmotic pressure of the body, an equilibrium which stabilizes the distribution of fluid between intercellular spaces and the blood stream. Actually the condition in the skin cannot be one of "equilibrium" but of a "steady state," for the continual loss of water from the skin by evaporation must mean a steady filtration of water from the blood stream through the peripheral tissues. A very small difference maintained between the mean capillary pressure and the colloid osmotic pressure would suffice to drive this steady filtration. It is easy to see how comparatively slight changes in either of these pressures might greatly reduce the filtration and hence the evaporative loss, since the permeability of the capillaries to water is very great. This

TABLE 4

DATE	ROOM TEMPERATURE	HUMIDITY	VAP. PRESS.	V.P. DIFF., SKIN TO AIR		EVAP. LOSS CALS./SQ.M/HR.		W_μ		PER CENT WETTED AREA	
				E	F	E	F	E	F	E	F
	°C.	*per cent*	*cm. Hg*								
6:20	32.4	72	26.0	1.62	1.62	39.2	44.7	24.2	27.6	85	97
6:21	31.65	54	18.7	2.20	2.38	32.0	20.0	14.5	8.4	51	29
6:22	31.34	57.5	19.5	2.08	2.12	14.3	40.6	6.9	19.1	24	67
6:23	32.92	64	23.7	1.79	1.80	32.2	29.2	18.0	16.2	63	57
6:24	25.65	50	12.7	2.26	2.22	9.8	18.3	4.3	8.2	15	29
6:25	23.4	50	10.7	2.21	2.34	9.2	10.0	4.2	4.3	15	15
6:26	23.3	53	11.3	1.83	2.32	7.4	8.4	4.0	3.6	14	13
6:27	23.3	60	12.8	1.94	1.96	11.75	8.8	6.0	4.5	21	16
6:28	23.3	62.5	13.4	1.88	1.95	10.5	9.4	5.6	4.8	20	17
6:29	23.3	64.5	13.8	1.99	1.98	11.2	12.7	5.6	6.4	20	22
6:30	23.3	61	13.1	1.97	2.05	8.2	9.5	4.2	4.6	15	16

might be the explanation of the abnormally low evaporative losses found in the initial stage of acclimatization to cold.

In experiment 3, colloid osmotic pressures were measured by Dr. J. Doupe and blood pressure measurements were available for the same experimental periods. These are given in table 5, which shows that there was a rise of colloid osmotic pressure in both subjects in the first three days of cold, a change which was maintained in subject F but subsided in E. Reliable measurements of capillary pressure were lacking, for these were not possible in the conditions of the cold where there is strong vasoconstriction. The difference between mean arterial blood pressure and colloid osmotic pressure might be taken as an indication of the direction of changes occurring in the filtration pressure. In spite of the complicated changes in mean pressure (table 5), this difference did show a fall to a minimum in the cold followed by a steady rise. The time relations

of its changes agreed well with those of the evaporative loss. The conditions of filtration of water from blood stream to tissues, though possibly not normally a limiting factor, probably does play a part in the changes seen in acclimatization to cold. Its rôle is apparently of more importance in non-basal than in basal conditions.

Radiation and convection. In experiment 4 direct measurements were made of the radiation and convection from the hand of the subjects at the same time each day.

The hand was placed within a closely wound cylindrical grid of wire, which had a high temperature coefficient of resistance. This grid, and a second control grid of similar construction placed near by, formed two arms of a Wheatstone bridge. The disturbance from balance of the bridge, shown by the deflection of a galvanometer,

TABLE 5

Mean arterial pressure (M.P.) and colloid osmotic pressure (C.O.P.) in millimeters of mercury

DATE	TEMPERATURE CONDITIONS	SUBJECT E			SUBJECT F		
		M.P.	C.O.P.	Diff.	M.P.	C.O.P.	Diff.
6:20.39	Warm	106.0	24.8	81.2	96.6	27.8	68.8
21	Warm	92.4	24.8	67.6	92.7		
22	Warm	86.7	25.9	60.8	82.2	28.3	53.9
23	Warm	88.7	24.1	64.6	75.9	26.1	49.8
24	Cool	86.4	24.7	61.7	76.7	27.5	49.2
25	Cool	85.0	26.4	58.6	84.7	30.6	54.1
26	Cool	77.2	26.9	50.3	76.3	29.2	47.1
27	Cool	81.2	24.5	56.7	76.4	30.0	46.4
28	Cool	81.0	25.3	55.7	88.3	30.3	58.0
29	Cool	89.0	24.7	64.3	87.8	30.7	57.1
30	Cool	97.6	25.0	72.6	84.6	31.0	53.6

was a measure of the heat received from the hand by radiation and convection. Evaporative heat was not included, since when the steady state was reached, in some five minutes, condensation of moisture on the wire of the grid was balanced by the evaporation to the outside air. Half-hour periods were used, and calibration in calories per hour was made by the substitution of an electrically heated "artificial hand" for the hand of the subject.

Figure 2 shows the results in this experiment. The 24 hour evaporative loss, expressed in the same units of calories per square meter per hour, is also plotted. The radiation and convection losses in the hot periods behave in general in the opposite way from the evaporative losses, so that there is no evidence of large changes in the total heat loss. In both subjects in the hot period there was an initial fall of radiation and convection from the hand followed by a progressive rise. In one subject, the course of this rise was resumed after the intervening two days of cold, in the

other a repetition of the sequence of fall and rise followed. A remarkable instance of the reciprocal relationship is seen in the first day of heat following the two days' cold for subject D. Here the evaporative loss was remarkably low for this temperature, while the radiation and convection losses were correspondingly high.

The changes in skin temperature in the heat of experiment 3 (fig. 1) are not significant, for here the temperature of the room was inadvertently lower on the second and third day. Progressive increases in finger blood flow found in experiment 2 and to be described in a later paper indicate

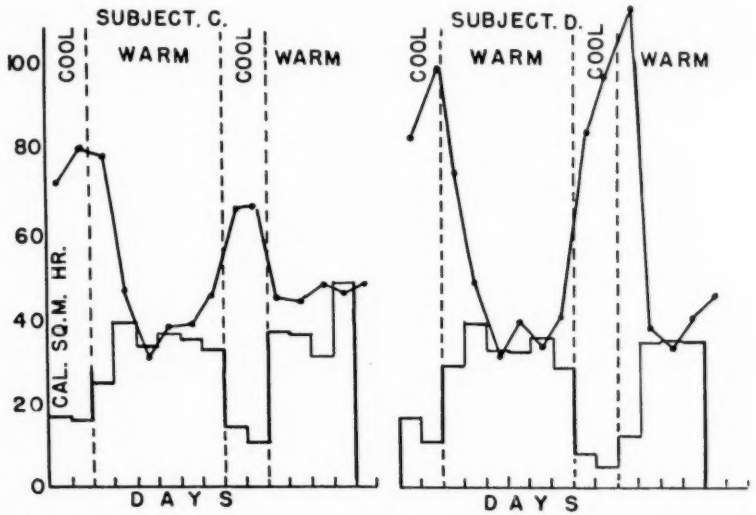

Fig. 2. Heat losses by evaporation (lower blocked curves) and loss by radiation and convection from the hand (upper curves) in experiment 4.

that there is in this area an eventual increase in radiation and convection during acclimatization to heat.

In acclimatization to cold, however, the situation is very different. The total heat loss cannot remain constant. As sweating is absent, both insensible loss and radiation and convection depend upon the skin temperature in a purely physical relationship. In the first three days of cold (expt. 3, fig. 1) the average skin temperature was falling as well as the evaporative loss; radiation and convection must also have decreased. Measurements of blood flow in the fingers in this and in experiment 2 support this conclusion, for flow decreased from abnormally high values on the first day to reach the normal low values of full constriction by the third day. Total heat loss in the first three days of cold must then have

steadily decreased. Subsequently average skin temperature, and presumably radiation and convection, remained constant or rose slightly while evaporation increased towards normal values. Acclimatization to cold evidently involves large changes in the total heat exchange as well as in its partition.

Storage of food in acclimatization to cold. The evidence just cited that in the initial stages of exposure to cold there is a fall of total heat production, which is also seen in the basal heat production (fig. 4), is in striking contrast to the fact that caloric intake showed an immediate and sustained increase in the same periods. There must have been a marked metabolic unbalance in these days. Additional evidence supporting this conclusion is found in the changes of body weight. Large changes in plasma volume were estimated following the changes in environmental temperature

TABLE 6

Results in grams

	CHANGE IN CONDITIONS									
	Cool to warm			Warm to cool						
	Experiment									
	1	1	4	1	2	2	2	2	3	3
	Subject									
	A	B	C	B	A	A	C	C	E	F
(a) Change in plasma volume	+403	+803	+742	−453	−390	−472	−213	−65	−890	−1430
(b) Change in weight	+438	+680	+297	−111	+179	−140	+767	+885	+143	−208
Difference between (a) and (b)	+35	−123	−445	+342	+569	+332	+980	+950	+1033	+1222

(Bazett et al., 1940). If there were no other changes, comparable alterations in body weight should occur. Table 6 gives the actual changes of weight that occurred between days on which the measurements of plasma volume were made. In acclimatization to heat the increases in plasma volume were accompanied by parallel increases in weight, some greater and some less than the plasma changes. In the acclimatization to cold the decreases in body weight were much less than the decrease in plasma volume and in some cases a weight increase accompanied a decrease of plasma volume. Loss of fluid is apparently to some extent balanced by an increase in the total solids of the body, possibly in the fat. Storage results because the increased appetite of the cold conditions is coincident in the first few days with a decreased total heat production.

Changes in fluid balance. The changes in temperature were accompanied

by very great changes in both intake of fluid by water drinking and in output by urine excretion. In the hot room the average water taken (all experiments, 47 subject days) was 1760 cc. per day, while in the cold room it averaged 450 cc. (40 subject-days). Calculation in one experiment of the total water intake, including that of the food and water formed by oxidation, gave averages of 3980 cc. in the hot and 2590 in the cold room. As adaptation to heat proceeded, the diminution in evaporative loss was reflected in a decrease in the intake of water (fig. 3).

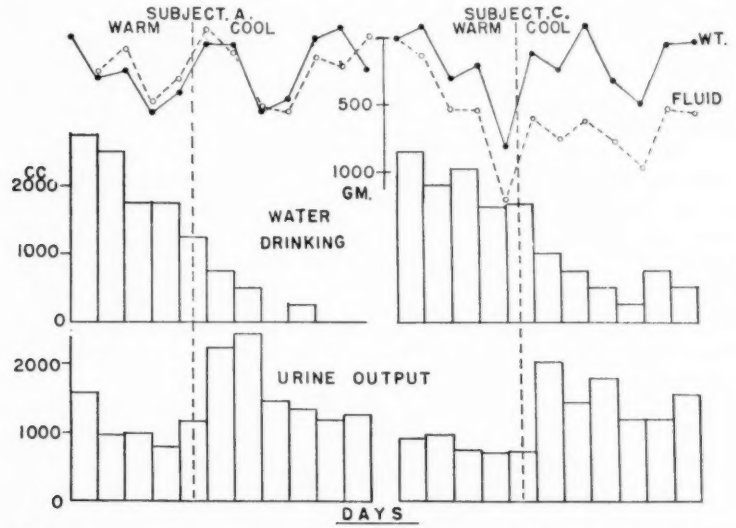

Fig. 3. Daily ingestion of water and urine output of the two subjects in experiment 2. The upper curves show the changes in morning weights, and in the calculated fluid retention.

Urine output in the hot periods averaged 850 cc. per day (all expts.), a value within normal limits. Upon the lowering of the temperature a large diuresis occurred in each case (fig. 3). In experiment 2 it did not develop in either subject until some 15 hours after the change of temperature, but reached its maximum rapidly. It is questionable whether it had completely subsided at the end of the experimental cold period. The average daily urine output in the cold periods, for all experiments, was 1600 cc.; maxima of over 2300 cc. were reached in some cases. This diuresis suggests that the slow adaptive changes, that occur when high climatic temperatures are changed to cold, are dependent upon a large change in the total volume of fluid in the body.

With complete data available of the food intake, it is possible to calculate the intake of water in the food, and again upon the assumption of complete metabolic balance each day, the water formed by oxidation of the food. Thus with a slight uncertainty as to the water content of the feces, a complete fluid balance may be struck. This has been done in the case of experiment 2 (broken lines in fig. 3). Critical analysis of the dependence of the calculation upon the assumption of metabolic balance reveals however that it is very little more reliable as an index of fluid balance than is the weight. The calculated retention of fluid is then an index of the retention, not of water alone, but of (water + 1.01 × fat + 0.99 × carbohydrate + 0.50 × protein). For example, the "oxidative loss" in the oxidation of 100 grams of fat amounts to a gain of weight of 7 grams causing an error in the estimate of evaporative loss. The water formed is 108 grams. If, therefore, 100 grams of fat were stored as such in the body instead of being oxidised, the calculated retention of fluid would be 101 grams in excess of the true amount. Since the weight is a measure of the retention of (water + fat + carbohydrate + protein), the laborious calculation of "fluid retention" gives values which differ from the mere changes in weight only in so far as they are less dependent upon protein balance.

Probably large fluid changes did occur in the process of acclimatization and were associated with alterations in the peripheral circulation, blood volume and in loss of water by evaporation. If, as has been deduced, much of the daily intake of food in the initial days of cold was not burned, but stored, the assumption of metabolic balance in the calculation of fluid leads to a much too great estimate of water formed. Thus the decreases of fluid in the cold were probably much greater than those shown in figure 3.

The fluid loss was accompanied by loss of chlorides though the data available are inadequate to calculate a chloride balance. During the diuresis to cold the salt excretion in the urine averaged 0.252 gram/square meter body surface/hour in experiment 2. The extra salt taken with the food (which was cooked without addition of salt) was 0.066 gram/square meter/hour. In the preceding warm days the urinary salt excretion had averaged 30 per cent lower, and the extra salt intake 30 per cent higher.

Basal metabolic rates and mean body temperature. Figure 4 shows the basal metabolic rates calculated from the oxygen consumption in the basal periods of experiment 3. There was a remarkable fall in the first few days of cold amounting to more than 15 per cent. In other experiments where sensations of cold were marked, there might be instead an initial increase in metabolism. This was followed by a progressive rise toward normal values at the end of the period. On the same graph the average temperature of the body tissues is plotted. This was calculated using the formula (0.65 × rectal temperature + 0.35 × surface temperature) introduced by one of us (Burton, 1935). The use of other but similar formulae (Hardy and DuBois, 1938) does not greatly affect the shape of the curve of average temperature. Assuming a Q_{10} of metabolism of 2.9 (Bazett et al., 1937) the expected values of metabolic rate were cal-

culated from the metabolism and average body temperature of the first day. These are plotted in figure 4 as crosses joined by a broken line. The intial decrease of metabolism in the cold was not far from that to be expected from the lowering of average body temperature. After the second or third day of cold, however, the metabolism progressively rose though average body temperature remained low. Such an eventual rise in the cold was seen in the other experiments, in which also there was some evidence of a slow decrease during the process of acclimatization to heat. In experiment 4 after the two days of cold had interrupted the heat, there was a progressive rise instead of the fall seen in the first period

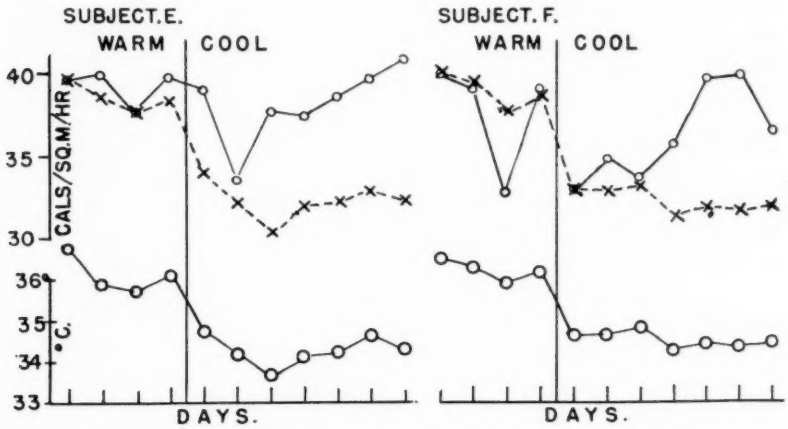

Fig. 4. Upper curves (solid lines) show basal metabolic rates, lowest curves (solid lines) the calculated average temperatures of the body. The crosses and broken curves show the metabolic rates calculated from the average temperatures and the initial metabolic rate on the assumption of a Q_{10} of 2.9.

of heat. The changes are suggestive, in their delayed onset and slow development, of the action of a hormone such as that of the thyroid gland. The action of thyroxin does not reach its maximum for some days (Canzanelli and Rapport, 1933) and there is much evidence in lower mammals of the stimulation of this gland in the cold (Schwabe et al., 1938). Evidence of seasonal variations in metabolism in man was also reported by Griffith et al. (1929).

Discussion and conclusions. Though the physical conditions of the four experiments were not exactly similar and the various subjects differed greatly in age and type, the many lines of evidence obtained do permit some general conclusions as to the alterations in heat exchange during the process of acclimatization. In an attempt to summarize the

various results, directly measurable or deduced, figure 5 has been drawn. It represents the results of an imaginary experiment made upon a non-existent "average" subject. The imaginary experiment suffered from no complicating physical or physiological factors of a non-essential character and the periods were long enough to achieve complete acclimatization. The curve of evaporative loss is deduced from direct measurements made in all the experiments. Radiation and convection was directly measured, for the hand only, in one experiment, and its direction of change deduced in others from the changes in skin temperature and circulation in the finger. The curve of the total heat loss is largely inferred from the

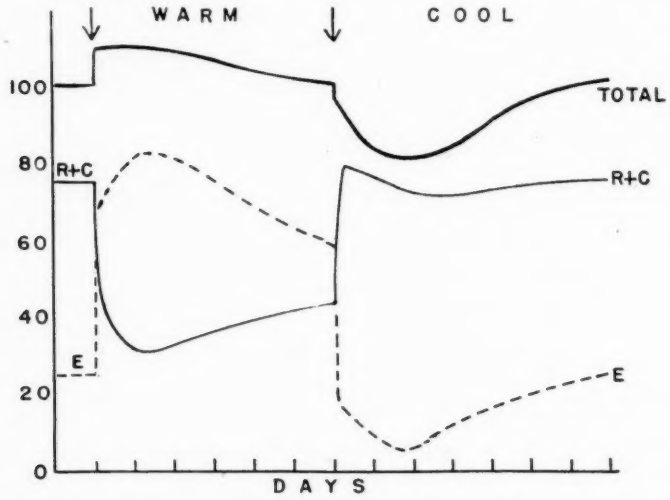

Fig. 5. Schematic diagram illustrating the slow changes in total heat exchange and its partition into loss by radiation and convection (R and C) and by evaporation (E) during acclimatization.

sum of losses by evaporation and by radiation and convection, but its shape agrees with that actually found for the basal metabolic rates in several of the experiments.

Changes in the total heat exchange are probably less in acclimatization to heat than to cold. In the heat an initial increase with the increased average body temperature is followed by a slow fall, possibly connected with a decreased thyroid activity. In the cold the immediate fall is followed for two or three days by a slow fall to subnormal values with a falling average body temperature. This is succeeded by a progressive rise, possibly connected with increased thyroid activity, though the average body temperature remains low.

Heat loss by evaporation may first rise in the heat, then progressively

fall, while loss by radiation and convection changes in the opposite manner. The eventual rise of radiation and convection loss is dependent upon an increased peripheral blood flow and skin temperature. Its maintenance, though possible by compensatory vasoconstriction, imposes less strain upon the organism after an increase in circulating blood volume has taken place.

In acclimatization to cold the changes in losses by evaporation and by radiation and convection follow courses that are parallel rather than opposite. When a cold follows a warm period full constriction of the peripheral vessels is not achieved for two or three days, and appears dependent upon the large diuresis which ensues. During these days skin temperature is falling and with it losses both by evaporation and by radiation and convection. A minimal evaporative loss is reached which is subnormal, partly because of the low skin temperature and partly because abnormal conditions for filtration of water through the skin may occur at this stage. Subsequently evaporative loss rises to normal values while radiation and convection remains constant or rises slightly.

The partition of heat losses changes in acclimatization to heat in such a way that eventually there is less dependence upon evaporation, here maintained at high values by sweating. In acclimatization to cold the opposite is achieved; radiation and convection losses are reduced.

The slowness with which these adaptations occur may be emphasized. Several days are required for their full development and when once established they are not easily reversed. For example, in experiment 4, after the two days of cold which separated two periods of heat, the evaporative loss of one subject in the first 24 hours of heat averaged only 21.3 gm./sq.M/hour, though on subsequent days it remained over 60 gm./sq.M/hour; a value to be expected at this temperature. The diuresis and the changes of weight also showed this property of "inertia" for they extended often into subsequent periods of changed temperature before showing reversal. A possible, though not necessarily the true explanation, might be modification by temperature of hormonal factors.

So many different physiological changes are involved in the process of acclimatization that it is not surprising that differences in the rate of these adaptations may lead to abnormal conditions in the early stages, such as the occurrence of subnormal evaporative loss in the first few days of cold. These conditions are probably to be regarded as incidental rather than as affecting the final result, which is the achievement of greater physiological "comfort" by modification of the modes of heat exchange.

SUMMARY

1. Data accumulated in four experiments in which the subjects remained for several days in an air-conditioned room kept at either relatively high or relatively low temperatures have been studied to determine the

changes in heat exchange and its partition in various channels during slow adaptation to the conditions.

2. Deep body temperature was maintained at normal levels. A slight shift with new conditions tended to be compensated as acclimatization proceeded.

3. Average caloric intake was significantly greater in cool than in warm conditions. Foods of higher caloric value per gram, which had a lower equivalent respiratory quotient, were chosen in the cooler conditions. Increased appetite plays a part in acclimatization to cold.

4. In acclimatization to heat, an increase in the total evaporative loss in the first one or two days is followed by a gradual reduction in later days. This is accompanied by changes in the loss by radiation and convection of a complementary nature, i.e., an initial fall followed by a progressive rise. The latter is associated with the increasing peripheral circulation described in another paper.

5. When cool climatic conditions follow a period of heat the evaporative loss, which may be supernormal on the first day, falls by the third day to a minimum value which is much below accepted standards. In the basal condition this is shown to be explained on physical grounds by the low value of skin temperature reached. The low values maintained throughout the day however are physiologically subnormal. The possibility of this subnormality being dependent on osmotic factors is shown. In later days of cold the evaporative loss rises towards normal values.

6. Radiation and convection is supernormal at the start of a cold period following heat, but falls with the evaporative loss in the first few days. This fall is associated with the slow decrease of skin temperature and peripheral circulation. In later days radiation and convection losses remain at the lower level or rise slightly.

7. The total heat exchange is deduced from the foregoing and by reference to basal values measured daily. In the heat there is evidence of a slow fall as acclimatization proceeds. In the initial days of cold there is a marked fall followed, after the second or third day, by a steady rise. Correlation of basal metabolic rates with average temperature of the body tissues suggests the operation of hormonal changes, possibly of the thyroid.

8. Since the increased caloric intake is not accompanied by an increased but by a decreased heat production in the first few days of cold, there is a marked metabolic unbalance on these days. Simultaneously there is a large diuresis continuing for several days and presumably a loss of body fluid, which is to some extent balanced by the storage of food in the body. Weight changes then are less than the changes of fluid content of the body.

9. Calculations of complete fluid balance are made but it is shown that these are as unreliable as the weight changes in the absence of metabolic balance.

10. It is concluded that the processes of acclimatization involve changes in both total heat exchange and its partition. In the heat radiation and convection losses eventually play a greater rôle, in the cold they decrease as adaptation proceeds. The maintenance of the new partitions involves less strain on the organism after changes of the fluid volume of the body have taken place.

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CLIMATIC EFFECTS ON CARDIAC OUTPUT AND THE CIRCULATION IN MAN

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Adaptation to climate has been shown to be associated with changes in blood volume (Bazett et al., 1940) as well as with alterations in heat exchange and in partition of heat loss (Burton et al., 1940). These are associated with cardiovascular changes. Previously the cardiovascular reactions to standing were found to differ according to the season of the year (Bazett et al., 1935), though such differences could not be correlated in any simple manner with the temperature at the time of the observations (Scott, 1936). The contrast between the earlier and later of these two sets of observations may have depended on climatic differences, for the former were obtained in 1933 which was conspicuous for a long period of continuous warm weather and the latter in 1934 during weather with irregular fluctuations.

The observations were made on subjects kept for 1 to 2 weeks in an air-conditioned room at either a high or comparatively normal temperature in the experiments already described in relation to blood volume (Bazett et al., 1940). In the course of these experiments the cardiovascular changes observed were similar to those found as the result of climatic changes. The character of some of the latter may be demonstrated by values obtained on two subjects in 1933. These values, many previously unpublished, are shown in figure 1. They represent repeated measurements of basal pulse rates and diastolic pressures on subject A of this paper throughout the summer, together with some obtained on subject 6 of the paper of 1935 in the early spring and fall. Continuous lines are drawn regardless of subject since the values shown were always almost identical on these subjects when they were examined at the same season. The mean values of the external temperatures in the periods of the experiments are also shown; for these we are indebted to the local bureau of the Department of Agriculture.

The onset of warmer weather resulted in a slight slowing of the pulse with a decreased change on standing. Diastolic pressure fluctuated, but showed a gradual increase in level. With the onset of colder weather the

pulse rate changes were reversed, but the rise in diastolic pressure was exaggerated and showed no reversal during the period of observations. Experiments in the controlled room confirm these observations (see table 1, 21) and demonstrate that the cardiovascular state is determined not only by the temperature conditions of the moment, but also by the history of the subject's exposure. Long lasting after-effects may be seen and the results of exposure to high temperature are not all immediately re-

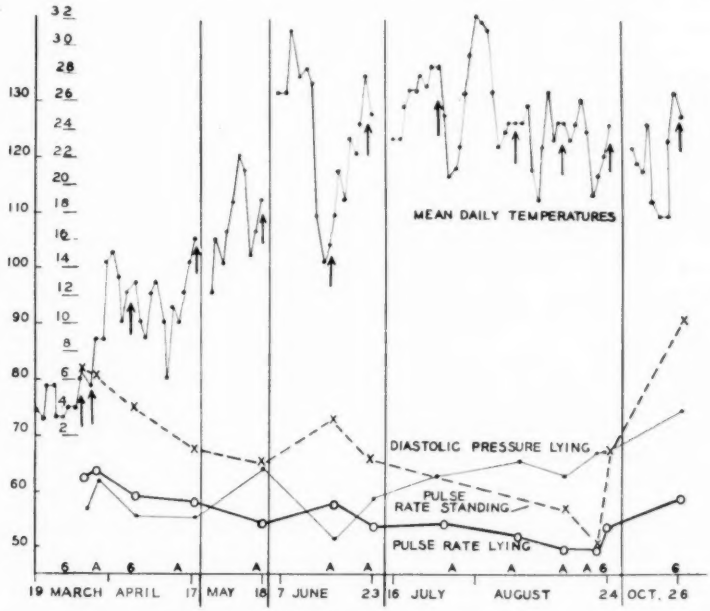

Fig. 1. Days of various periods in 1933 are plotted as abscissae, and from above downwards mean daily outdoor temperatures, standing pulse rate X------X, lying pulse rates O—— O and lying diastolic pressure .——. Times of observation are indicated on the temperature record by arrows. The subjects utilized on each occasion are shown by their symbols (*A* or *6*) below.

versed by exposure to a low one; some of the immediate effects of the changes may be additive.

METHODS. The conditions of the experiments have already been stated in the earlier papers. Additional methods of examination employed were measurements of cardiac output by acetylene both with the subjects lying down (experiment 1 few observations and experiment 3) and with the subjects standing (experiment 2) both basal, and non-basal in the afternoon (some 2½ to 3 hours after a light lunch). Estimates of cardiac output were also made from measurements of blood pressure and pulse wave velocity with the subjects both lying and standing by the method pre-

viously described (Bazett et al., 1935). The measurements of blood pressure represent averages of some 4 or more measurements on optical records. In experiment 1 some measurements of blood pressure were also made by auscultation. In experiment 2 venous pressure measurements were made daily by direct puncture of the veins at the elbow; the subject was lying down and the arm was abducted through 30°. Values are expressed relative to the mid point of the anterio-posterior diameter of the thorax opposite the costal angle.

Measurements of blood flow in the finger were made when the subject had been lying down for at least 20 minutes. The method was that described by Burton (1939), and depended on the optical recording of increases in finger volume when venous outflow was suddenly obstructed. Owing to the rhythmic fluctuations in flow common in the fingers some 20 determinations were made at intervals of 10 to 20 seconds.

The routine for measurement of cardiac output by acetylene followed standard lines; samples were drawn after approximately 12 and 18 seconds' rebreathing. In experiment 3 an additional third sample was taken. A standard gas mixture (N_2 70 per cent, O_2 25 per cent and CO_2 5 per cent) was employed to dilute the acetylene and this facilitated the procedure. Mixtures were employed which gave ultimate concentrations of 10 to 15 per cent C_2H_2 and 12 to 16 per cent O_2. The routine for blood pressure and pulse wave velocity measurements also followed the technic previously described and sternal pulsations were utilized (Bazett et al., 1936) to time cardiac expulsion. A heavy x-ray tube stand was employed to hold in position all tambours applied to the trunk (with the subject lying down) and this proved to be a great convenience. Some of the records of blood pressure and of pulse wave velocity with the subjects standing were inadequate since this procedure was commonly conducted during the inhalation of oxygen and was not allowed to interfere with or delay the acetylene rebreathing procedure. The latter ended the period of standing some 14 to 16 minutes after assumption of the vertical position. The diastolic criterion was also sometimes indefinite, even with the subject lying down, in the younger subjects in a warm room.

RESULTS. The changes in temperature were accompanied by definite changes in the cardiovascular system which progressed steadily, and showed no simple relationship to the temperature at the moment. The direction of change often became reversed with time, i.e., warmth induced an initial increase in both pulse rate and blood pressure levels which was followed by a later decrease, while return to cool conditions caused a fall followed by a rise. These changes are illustrated by charts and in table 1. In this table the averages of subject averages are given, for preliminary cool days and for the warm and cool conditions. In the latter the mean values for the first two and last two days are given as well as the means for all days. The presence of trends is thus demonstrated. These average values are somewhat complicated by variations in routine and the occasional absence of data on one or the other subject. Where the inclusion of a different number of subjects makes the average value misleading the value is bracketed; where the mean values represent changes consistently indicated by the individual data, the figures are emphasized by insertion in italics.

TABLE 1

OBSERVATIONS	CONTROL DAYS	WARM DAYS			COOL DAYS		
		First two days	Average of all days	Last two days	First two days	Average of all days	Last two days
1. O_2 (ml./m²/min.) lying a.m.		*132.8*	*128.8*	*124.2*	*120.8*	*126.8*	*132.2*
2. O_2 basal standing a.m.		*153.9*	*147.1*	*141.7*	*140.5*	*142.8*	*146.5*
3. O_2 lying p.m.		142.8	141.5	140.2	142.5	140.0	140.2
4. O_2 standing p.m.		161.4	164.4	169.2	159.0	167.1	167.8
5. Art-Venous O_2 diff. lying a.m.		54.5	*55.2*	55.8	60.8	*59.6*	59.0
6. Art-Venous O_2 diff. ml./l. standing a.m.		74.4	*77.1*	78.3	*80.5*	*85.9*	*88.0*
7. Art-Venous O_2 diff. standing p.m.		86.8	*84.6*	81.3	*89.3*	*92.4*	*95.9*
8. Cardiac index (C_2H_2) lying a.m.		*2.47*	*2.37*	*2.27*	*1.92*	*2.10*	*2.22*
9. Stroke Vol. ml./m² (C_2H_2) lying a.m.		32.8	33.4	33.9	31.8	33.3	36.8
10. Cardiac index (C_2H_2) standing a.m.		2.06	*1.96*	1.88	*1.80*	*1.65*	*1.66*
11. Stroke Vol. ml./m² (C_2H_2) standing a.m.		26.3	*26.0*	26.2	*27.8*	*24.5*	*22.9*
12. Cardiac index (C_2H_2) standing p.m.		*1.86*	*1.95*	*2.08*	1.78	*1.80*	1.77
13. Stroke Vol. ml./m² (C_2H_2) standing p.m.		*20.8*	*22.5*	*23.8*	*22.3*	*20.8*	*20.6*
14. Cardiac index lying calculated a.m. and p.m.	1.88	2.20	2.08	1.96	2.00	1.92	1.90
15. Pulse rate lying a.m. and p.m.	56.8	61.5	60.1	59.0	59.3	59.0	60.0
16. Pulse rate standing a.m. and p.m.	87.3	89.7	*85.7*	*82.9*	*80.5*	85.5	*85.0*
17. Systolic pressure (mm. Hg) by pulse lying a.m. and p.m.	115.4	*119.3*	*114.4*	*110.4*	*113.4*	*115.0*	*122.2*
18. Systolic pressure by pulse standing a.m. and p.m.	126.9	127.0	125.1	123.5	126.2	125.6	130.8
19. Lateral systolic press. lying a.m. and p.m.	109.7	*112.6*	*108.5*	*105.0*	*109.7*	*110.2*	*115.7*
20. Lateral systolic press. standing a.m. and p.m.	122.6	121.2	119.5	118.6	122.7	122.4	127.9
21. Diastolic press. lying a.m. and p.m.	67.6	65.2	*64.3*	63.9	*68.8*	*69.3*	*77.8*
22. Diastolic press. standing a.m. and p.m.	93.6	90.8	86.5	83.1	*88.0*	*91.0*	*95.4*
23. Mean pressures lying a.m. and p.m.	89.0	88.6	86.4	84.7	*88.6*	*89.9*	*97.5*
24. Mean pressure standing a.m. and p.m.	107.7	105.6	*102.6*	100.3	*105.1*	*106.8*	*111.6*
25. Pulse pressure lying a.m. and p.m.	42.1	47.4	44.2	41.1	40.9	40.9	37.9
26. Pulse pressure standing a.m. and p.m.	29.0	*30.4*	*33.0*	*35.5*	*34.7*	*31.4*	(32.5)
27. Effective resistance (R) lying a.m. (est. by C_2H_2).		114	113	113	128	120	117
28. Effective resistance (R) lying a.m. and p.m.	142	121	126	133	*134*	*142*	*159*
29. Effective resistance (R) standing a.m. and p.m.	191	*188*	*167*	*152*	*171*	*184*	*220*

TABLE 1—*Concluded*

OBSERVATIONS	CONTROL DAYS	WARM DAYS			COOL DAYS		
		First two days	Average of all days	Last two days	First two days	Average of all days	Last two days
30. Effective resistance (R) standing a.m. and p.m. estimated by cardiac indices by C_2H_2		181	165	146	200	197	204
31. Pulse wave velocity (m/sec.) I. Lying a.m. and p.m.	3.94	3.97	3.93	3.95	3.74	3.76	(3.83)
32. Pulse wave velocity I. Standing a.m. and p.m.	(3.08)	3.25	3.21	3.20	(3.34)	3.30	(3.13)
33. Pulse wave velocity II. Lying a.m. and p.m.	5.58	5.86	5.81	5.72	5.72	5.69	(6.20)
34. Pulse wave velocity II. Standing a.m. and p.m.	(7.31)	8.06	7.64	7.45	7.41	7.37	7.21
35. Pulse wave velocity III. Lying a.m. and p.m.	6.68	7.02	6.89	6.59	6.62	6.81	6.75
36. Pulse wave velocity III. Standing a.m. and p.m.	(7.84)	7.06	7.43	7.95	8.44	7.72	(7.68)
37. Pulse wave velocity IV. Lying a.m. and p.m.	9.52	8.31	8.44	8.55	9.13	9.22	(8.51)
38. Pulse wave velocity IV. Standing a.m. and p.m.	14.85	13.79	*13.90*	13.94	13.69	*14.49*	15.34
39. Distensibility of central arteries lying a.m. and p.m. (ml./m²/mm. Hg change in pressure)	0.543	0.531	0.534	0.527	0.564	0.569	(0.531)
40. Distensibility of central arteries standing a.m. and p.m.	(0.672)	0.609	0.596	0.545	0.570	0.599	(0.514)
41. Distensibility of periph. arteries lying a.m. and p.m.	0.120	0.152	*0.155*	0.166	0.127	*0.129*	(0.143)
42. Distensibility of periph. arteries standing a.m. and p.m.	0.062	0.076	*0.072*	0.067	0.066	*0.064*	(0.059)
43. Venous pressure (cm. of water) a.m.		10.9	11.2	11.8	13.1	12.3	12.0
44. Mouth temp. a.m.	36.14	*36.78*	*36.73*	*36.64*	36.29	*36.39*	36.37
45. Mouth temp. p.m.	36.35	*37.02*	*36.95*	*36.85*	36.49	*36.49*	36.54
46. Rectal temp. p.m.	36.89	*37.29*	*37.25*	*37.18*	36.90	*36.75*	36.61

Values given represent all subjects for columns 44 and 45, subject A experiments 1 and 2 for column 46; subjects C, E and F experiments 2 and 3 in column 1; subjects A and C experiment 2 in column 2, 4, 6, 7, 10 to 14, 30, 39 and 43; subject A experiment 2 only in column 3; subjects E and F experiment 3 in columns 5, 8, 9 and 27 and subjects A, B and C experiments 1 and 2 for all other columns.

Cardiac output. Data obtained *with the subject lying and basal* in the summer experiments (2 and 3) are shown in figures 2 and 3. The data of experiment 2 were obtained by calculation from the blood pressures, those by experiment 3 by acetylene. The greater irregularity of the values for cardiac output in the former may depend on the greater chance errors of the method; the general characteristics of the changes are similar. The data obtained by acetylene in experiment 3 also appear in table 1, columns 5, 8 and 9; comparison of figure 3 with the columns will allow an estimate of the minimization of progressive changes by the tabular method of presentation. The responses of the eldest and youngest subjects (figs. 2

and 3) were similar, except that an initial period of increasing cardiac output in the warmth was observed at the start of experiment 2. This

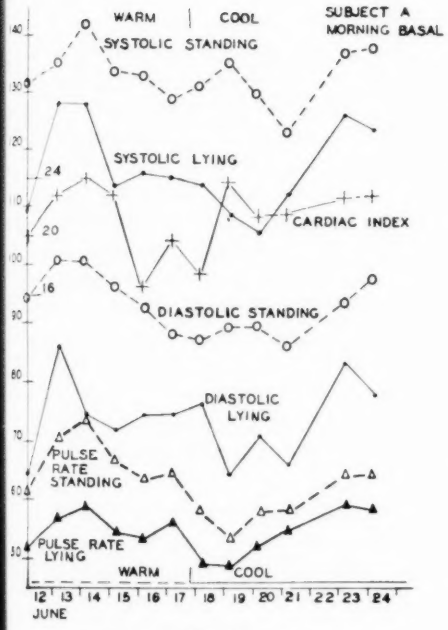

Fig. 2

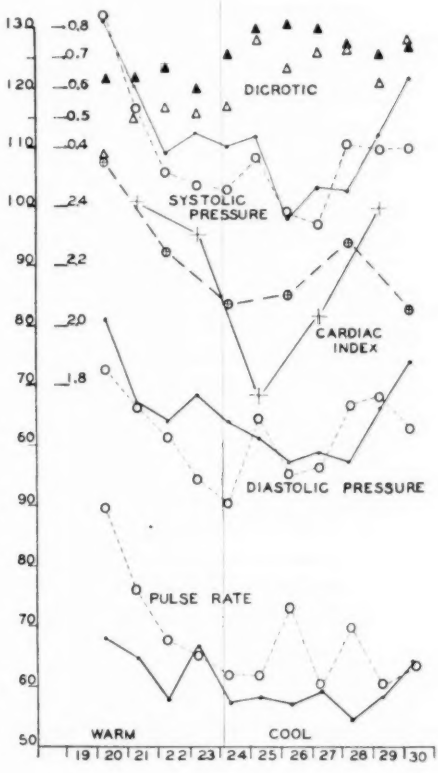

Fig. 3

Fig. 2. The changes in blood pressure and pulse rate in subject A in the summer experiment are indicated as ordinates with the dates of observations as abscissae. The cardiac indices obtained by calculation from blood pressure and pulse wave velocities with the subject lying are also shown as crosses.

Fig. 3. The days of June 1939 are shown as abscissae, pulse rates diastolic and systolic pressures as ordinates. Data were obtained in experiment 3 on subjects E and F. The former is shown in continuous lines, and closed circles; the latter is shown in dotted lines and open circles. The cardiac indices (lying by C_2H_2) are also shown as crosses and ringed crosses. Above, the shape of the pulse curve is indicated in triangles which give the ratio of the height of the dicrotic wave to that of the whole pulse (closed triangles for E and open for F).

probably depended on a lesser degree of summer acclimatization at its start. Changes in cardiac output under these conditions were a gradual

increase to slightly supernormal levels in acclimatization to warmth, followed by a gradual fall to normal levels, and in the cold initial subnormal levels followed by a gradual return to normal. These changes may be compared with those in blood volume and oxygen consumption. Graphs indicating the changes in both of these for experiment 3 have been given in previous papers. Reductions in cardiac output in the cold exceeded those in oxygen consumption, so that the A-V oxygen difference was increased relative to that in the warmth (table 1, 5). There is some evidence of slight progressive changes in the oxygen utilization both in the warmth and cold.

Estimates of *cardiac output with the subjects lying down but not basal* (late afternoon values) were all derived by calculations from blood pressure readings. They are combined in table 1, 14 with estimates made by this method in the basal state. The changes during acclimatization to warmth so measured were the same as those already described. There was a gradual reduction in cardiac output. In the cold no progressive increase with acclimatization was observed of the type seen in experiments 2 and 3. Whether this difference depended on the method employed for measurement, on the season of the year or on some other factor is uncertain. There is no doubt that the reactions to cold were variable. Excessive sensations of cold might stimulate metabolism so that oxygen usage might be initially increased rather than reduced. Though called normal, cardiac outputs may have been influenced by such a metabolic response to cold.

Aberrant changes in cardiac output might also be seen in the warmth if the temperature was high enough to cause dehydration. Such dehydration developed in subject C in experiment 2 and was associated with an increased pulse rate. Cardiac output as estimated from blood pressures with the subject lying showed great fluctuations from day to day. The cardiovascular reactions to standing and cardiac output in the standing posture (see later) were also modified. Whatever the real changes may have been under such conditions, the general description of acclimatization to warmth that has been presented can only be assumed to hold in the absence of excessive exposure and dehydration. Nor can the description be expected to hold unless temperatures are maintained uniform for several days. When a period of warmth was interrupted by one or two days of cold as in experiment 1 (subject A) and experiment 4, the behavior of the subjects to the second period of warmth differed from that seen in the first exposure. The reactions to such changes are complex and will be left for future analysis.

The stroke volumes observed with the subjects lying down did not vary greatly. Those determined by acetylene are shown in table 1, 9. Those determined by calculation may be obtained by dividing column 14 by column 15; the mean values for the warm conditions was 34.6 and for the cold 32.6 m./square meter.

Cardiac outputs with the subjects standing showed much more marked differences according to the climatic conditions than did those with the subjects lying down. The data are presented in table 1 columns 6, 7 and 10 to 13. These were all derived from data obtained in experiment 2, where daily measurements were made with acetylene on both subjects both under basal and non-basal (afternoon) conditions. Of the measurements made 42 appeared to be valid, and therefore warrant statistical analysis. These data are consequently given with their standard deviations in table 2, though 4 observations made in the transitional period of change from warmth to cold have been excluded. The rates of adjustment of different subjects in the transitional stages are apt to vary and consequently greater irregularities are found.

In warm rooms to which the subjects are acclimatized there is little reduction of cardiac output on standing. If subject C who showed some symptoms of dehydration be excluded the values are even higher, for subject A gave a mean value of 2.0 ± 0.24 basal, and 2.0 ± 0.09 (non-basal) liters/sq. m./min. in the warmth. The data agree therefore with

TABLE 2

Cardiac outputs standing (C_2H_2) in experiment 2

MORNING BASAL		AFTERNOON BASAL	
Warm	Cool	Warm	Cool
9 observations	11 observations	10 observations	8 observations
CI 1.95 ± 0.24	1.63 ± 0.13	1.95 ± 0.15	1.79 ± 0.17

the values originally reported by Grollman (1928) who claimed that standing was accompanied by little or no reduction of cardiac output. The data obtained under cool conditions give on the other hand an average for both subjects of 1.63 ± 0.13 under basal conditions; this value is entirely in agreement with the bulk of the literature on the subject. Analysis of the data using Fisher's t values (Fisher, 1932) indicates that the probability of the differences between warmth and cold being due to pure chance is less than 1 in 100 for the basal figures, and less than 1 in 20 for the non-basal. These differences must be considered significant.

The degree of reduction on standing may be approximately estimated by comparing the basal values obtained by calculation from blood pressures in experiment 2 on subject A lying down with those obtained on the same subject standing by acetylene. The values are for the warmth a reduction from 2.09 ± 0.28 to 2.00 ± 0.24 (5 per cent) and for the cold (excluding the first day) one from 2.23 ± 0.10 to 1.60 ± 0.14 (28 per cent).

A series of observations made on successive days demonstrates that the fluctuations in output are mainly due to physiological variations in the subject and do not depend on chance experimental errors. The changes

progress normally in a regular manner and the accuracy of acetylene for comparative measurements appears high. It has already been stated that subject C showed symptoms of dehydration in experiment 2. The actual daily estimates of cardiac outputs standing for this subject are shown in figure 4 together with the morning weights and ratings of physical fitness in the afternoons. In the basal state in the morning cardiac output progressively decreased in the warmth and reached relatively low values at the time of the greatest weight loss. These low values were associated with relatively low fitness ratings in the afternoon. Yet cardiac outputs measured in the afternoon a few minutes after the completion of the fitness tests showed progressively increasing values each day in the period of warmth with a maximum on the day of minimal fitness. This high cardiac output was attained by a fast pulse (114 per min.) and a relatively low stroke volume. No fitness tests were made on this subject in the morning.

The indication that in a series of daily observations the changes in cardiac output in the morning may be the exact opposite of those observed in the afternoon was not unique. Incomplete data obtained on subjects A and B in experiment 1 (by different methods in the morning and afternoon) gave indications of the same differences, though no such difference was observed in A when more fully adapted to heat in experiment 2. As will be shown later similar discrepancies were also seen when fitness tests were made both in the morning and afternoon.

The abrupt change from warmth to cold gave variable responses. Most subjects showed an initial stage when the cardiac output standing equalled a normal lying value (see values for C shown in fig. 4). Cardiac outputs with the subject lying were also possibly slightly increased. There was variation in the time of occurrence of this stage in different experiments.

Pulse rates and blood pressures. The changes in pulse rate and blood pressure were complicated when dehydration effects were combined with partial adaptation to heat, as was the case in subject C in experiment 2. Afternoon data obtained in the non-basal state differed also to some extent from those obtained under basal conditions. The data obtained on subjects A, E and F in the summer experiments (2 and 3) are shown in figures 2 and 3. These data were all obtained under basal conditions and in the absence of evident dehydration. The changes will be seen to correspond closely with those of the composite data of experiments 1 and 2 shown in table 1, columns 15 to 26. Other data on subjects A and B in experiment 1 are shown in figure 5, where the diastolic pressures with the subjects lying down, and the pulse rates with the subjects standing are shown, for morning basal conditions as well as for the non-basal conditions of the afternoon. There is a marked contrast between the standing pulse rates of the mornings which became in both subjects faster for the

first 2 or 3 warm days, and those of the afternoons which began to slow on the second day and in subject B became much slower than those observed before the experimental period. The consequent effects on calculations of physical fitness will be discussed.

The general effects observed in the warmth were initial increases in the *pulse rates*, both lying and standing and usually in the difference between them (figs. 2, 3 and 5 and table 1, 15 and 16) followed by a reduction of

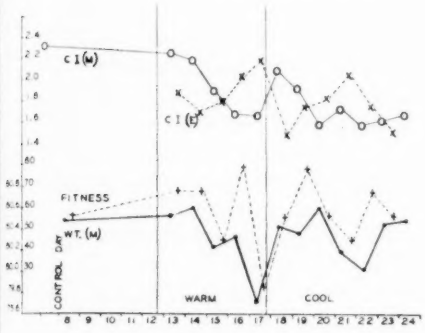

Fig. 4

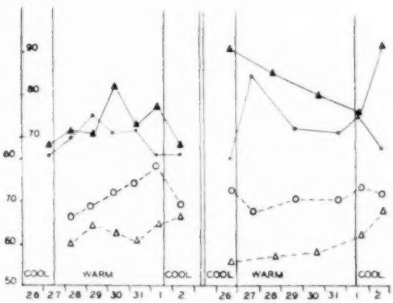

Fig. 5

Fig. 4. Dates of the summer experiment (June 1938) are indicated as abscissae, and as ordinates cardiac outputs per square meter of body surface with the subject standing basal in the mornings as CI (M) and in the afternoon, resting but not basal, as CI (E). All the observations were on subject C. Below these also as ordinates are plotted the cardiovascular fitness of the subject in the afternoon +-----+, as estimated by the Schneider standards from changes in pulse rate and systolic blood pressure on standing, and the basal morning body weight ·——· Scales are given to the left for body weight in kilograms, to the right above for cardiac indices and below for percentage fitness.

Fig. 5. Changes in the standing pulse rate (·——· for subject A, and ▲——▲ for subject B) for the winter experiment are shown as ordinates with the dates of December 1937 and January 1938 as abscissae. Diastolic pressures with the subjects lying (O-----O for subject A, and △——△ for subject B) are also shown below the pulse rate values. The graphs to the left represent observations made on these subjects in the morning under basal conditions, those to the right in the afternoon with the subjects resting but not basal.

all these values. In the cold following warmth all these values continued at first to fall still further, but the changes were reversed after several days. The data show no abrupt changes at the time of the abrupt change in temperature (figs. 2 and 3).

Diastolic pressure with the subject lying down might show an initial marked rise (fig. 2) or a fall (fig. 4 afternoon data and table 1, 21) when the environmental temperature was raised; the pulse rate, as might be expected, influenced the level attained. The level later fell and remained

low but tended to rise at the end of the warm period (marked changes in the reverse direction in subject C with dehydration mask this terminal effect in the table). In the cool there was an initial fall followed by a rise (see figs. 2 and 3). In general the changes both in pulse rate and diastolic pressure followed closely those shown as seasonal changes in figure 1. Diastolic pressure with the subject standing in the warmth first rose, then fell with adaptation; this fall appeared correlated with improved cardiac output and the need of less vasoconstriction for the maintenance of the circulation (table 1, 22, 10 and 11, 29 and 30). The opposite change was seen in the cool (fig. 2), though the initial fall might be slight, short-lived, or absent. The net effect of cold following warmth as compared with control values obtained before the experimental changes in temperature were a marked rise in diastolic pressure to a level considerably above the control level, a marked reduction in the acceleration of the pulse rate due to standing, with a lying pulse rate which was usually above the control level. Such changes disappeared slowly.

Systolic pressures and mean pressures with the subject lying showed less consistent changes but also usually rose at first in the warmth and then fell; they showed the reverse changes in the cold. Here too the ultimate pressures considerably exceeded control levels. With the subject standing the changes were much the same.

Shape of pulse curve. Not only did the pressures change in a consistent manner but also the shapes of the pulse curves. With decreased cardiac output and increased peripheral resistance the dicrotic wave was higher relative to the pulse curve as a whole. In figure 3 the position of the dicrotic wave is shown by plotting the ratio of the difference between the dicrotic wave pressure and the diastolic pressure to the difference between lateral systolic and diastolic pressures. The uniformity of the changes in the two subjects is obvious.

Venous pressure. The average values of the venous pressures in the two subjects of the second experiment are given in table 1, 43. There may have been a gradual rise in the warmth, with a further rise in the initial exposure to cold but the differences were slight, and the changes inconsistent. Both subjects however gave the highest values on the first day of exposure to cold. For the most part the pressures observed were approximately those required to raise the blood in the arm to the level of the manubrium sterni (see Doupe et al., 1938) and slight variations in the degree by which this pressure was exceeded were hard to measure.

Effective peripheral resistance and elasticity of the vessels. The distensibility of the large arteries was estimated from pulse wave velocity measurements, namely, PWV I from the heart to the subclavian, II from the subclavian to the femoral, III from the subclavian to the brachial, and IV from the femoral to the dorsalis pedis. The mean values of these pulse

wave velocities are given in table 1, 31 to 38. Estimates were made usually from measurements of 5 to 10 pulse cycles for each value in each subject, except where the subject was standing when records might be incomplete. In column 39 to 42 the effective distensibilities of the vessels have been calculated in terms of the *absolute* increase in volume of the larger central vessels and of the smaller peripheral vessels per millimeter mercury increase in pressure per square meter of body surface. The values given are arbitrary values calculated on the assumed diastolic volumes of the vessels concerned for these subjects (see Bazett et al., 1935); they have been expressed in this form in relation to surface area to reduce the effects of individual differences in size and age. The effective peripheral resistances (table 1, 27 to 30) have been calculated from the relationship of mean pressure (estimated by graphic integration of the brachial pulse curves) to cardiac output per square meter as estimated from the pulse pressures and distensibility values, except where otherwise stated.

It may be noted that, as adaptation to warmth develops and cardiac output is more adequately maintained, the necessity for intense vasoconstriction during standing becomes less. The opposite change is seen on adaptation to cold where cardiac output becomes reduced to a greater extent on standing and the necessity for intense vasoconstriction becomes greater (see table 1, 29 and 30 and compare with table 1, 10 and 12).

The estimates of cardiac output from pulse pressure measurements appear to be valid and free from serious systematic errors as judged by the close agreement of columns 29 and 30 of table 1. Any such absence of systematic errors in the complex conditions of standing can only be established on experimental evidence and previous evidence of this sort has been slight (Bazett et al., 1935). The agreement between columns 29 and 30 should not be taken to indicate that chance errors are also small in applying blood pressure analysis to the conditions of standing. The experimental observations which gave the data of these two columns do not always belong to the same experimental days. Actually there were 13 sets of observations in which direct comparison of the two estimates of cardiac output was possible. The mean discrepancy of the calculated values from those obtained by acetylene was $\pm$ 18 per cent of the latter. The average of the calculated values was also 13 per cent above that by C_2H_2. Artefacts are hard to avoid in pulse records obtained with the subjects standing, and accurate measurements are difficult. The difference between the two averages need not indicate any systematic error in either method. The circulatory state is not stable, and the calculated estimates represent conditions after standing for some 6 minutes, those by acetylene after standing for some 12 minutes.

Cardiovascular fitness. Estimates of fitness according to the scale described by Schneider (1920) was possible from the measurements of blood pressure and pulse rate in the lying and standing positions with a maximal possible score of 9/9. There was an initial decrease in fitness on exposure to warmth followed by an increase to one above that seen on

control days. In a succeeding cold period fitness initially improved still more, but later decreased. The standards of the scale are not adequate to distinguish small changes. Thus comparison of the average values on subjects A, B and C in table 1, 15, 16, 17 and 18, indicates an initial score of 7/9 on the control days, of 5/9 on the first two days of warmth, of 8/9 for the rest of the experiment, till the middle of the cool period when it returned to 7/9. A progressive improvement is, however, distinguishable throughout the exposure to warmth and the earlier part of the cold. Thus the *percentage* increase in pulse rate on standing for the 7 grouped periods is 54, 46, 40, 43, 36, 45 and 42. The data on seasonal changes shown in figure 1 give some indication that the effects would have been still greater if exposure to warmth had been more prolonged. Similar evidence of progressive changes in fitness is found in the magnitude of increase in blood pressure on standing (table 1, 17 and 18).

The improvement in fitness in prolonged exposure to warmth is lost in the presence of dehydration. This may be seen in data on subject C shown in figure 4, when the fitness and weight changes follow similar curves. Usually there was a close agreement between the fitness rating and the stroke volume of the subject when standing, but in view of the large effect of standing pulse rate on the estimate of fitness, this correspondence may not be of great significance.

Where fitness tests were made on the same subject both in the morning and afternoon, strange contrasts were seen in the changes developing from day to day. Thus observations in the afternoon might indicate a daily increase in fitness when those in the morning showed a decrease. The improvement in fitness in the afternoon appeared to develop earlier in exposure to warmth than did that shown in basal morning tests. The actual fitness ratings of the afternoon were, however, lower than the basal values. The variations in standing pulse rates observed on subjects A and B in experiment 1 both in the morning and afternoon are shown in figure 5.

Fitness tests appear capable of demonstrating changes in physical fitness in the individual. They do not, however, enable comparisons between individuals. By such tests A was the fittest subject, but he showed marked edema of the feet in early exposure to warmth during the winter and more mental confusion and inaccuracy than the other subjects.

The high pulse rates observed with the subject standing in the cold and the consequent low fitness ratings indicate that the low cardiac outputs observed under these conditions probably result from a circulation strained by an inadequate venous return.

Peripheral flow in the fingers. Peripheral flow in the fingers was measured by Dr. A. C. Burton daily in experiments 2 and 4 in the afternoon and in 3 in the morning with the subjects basal. The results obtained

on subjects A and C in experiment 2 are shown in figure 6. In experiments on normal subjects with heating pads applied to the legs, the maximal values for flow in the fingers has been 80 to 90 ml./min./100 ml. of tissue, with sustained averages rarely exceeding 80. This normal maximal level was present in C and exceeded in A on the first day of exposure in the warm room, though sweating was not obvious. Both the maximal

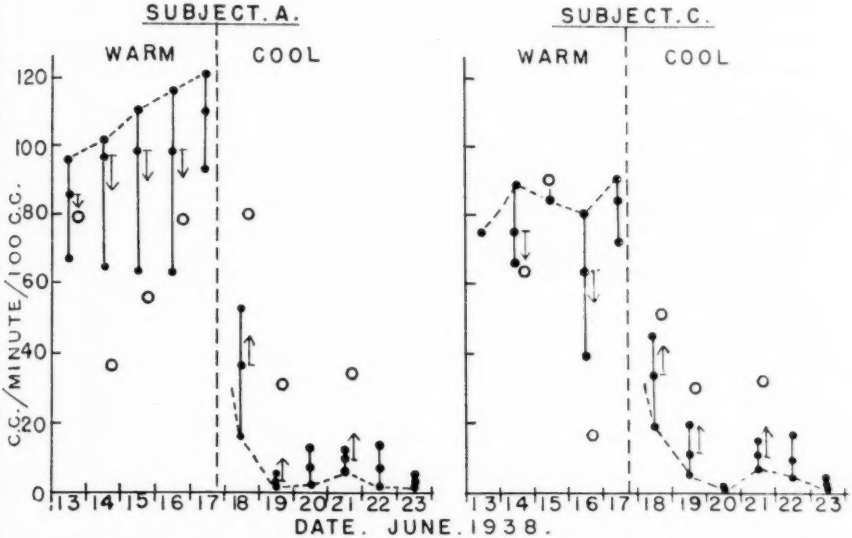

Fig. 6. Blood flows through the fingers are plotted as ordinates against the days of observation as abscissae. Average, maximal and minimal values are indicated and dotted lines are drawn through the maxima in the warm and through the minima in the cold period. The average rates of flow observed when the legs were warmed are indicated by open circles, and the arrows indicate the change from the average value before heating.

and average values for A rose steadily to much higher values in the five days of the hot period though the room temperature remained constant.

There is some uncertainty in the values shown for C in the hot room, as here the vessels showed so little distensibility that the total rise of volume upon occlusion was insufficient to allow accurate measurements. This difficulty was not present in the measurements made on subject A, nor in those on C in a cold room.

In the warmth the few reliable observations that could be obtained on C showed little tendency to rise but rather a sharp fall on the 4th day on which loss of weight occurred. In the cold the remarkable feature in this and other experiments was that on the first day, some 18 hours after the

temperature had been changed, the average flow in both subjects remained far above the normal constriction level (which is from 1 to 2 ml./min./100 ml. tissue). No single observation approached such a level. Only on the 2nd day of cold for A and the 3rd for C was full constriction apparently possible. (It may be noted that reduction of blood volume appeared to be more rapid in A than in C.) The development of minimal flow coincided in time with that of minimal evaporative loss (see Burton et al., 1939). Sensations of temperature were maximal in the early stages of cooling when the rate of change of room temperature was greatest; lack of sensory stimulation does not provide an adequate explanation.

Since the maximum flow is normally obtained by heating the subject, in experiment 2 the subjects were also wrapped in blankets and treated by the standard technic of heating the legs for 30 minutes. This produced violent sweating, but induced an abnormal effect on blood flow. Instead of the increase in flow that is normally obtained, there was a decrease. This decrease is shown in figure 6; it was at first small in subject A, became very great on the second day and thereafter steadily diminished. In subject C it was relatively very great on the day of loss of weight. It would appear that the circulation was unable to maintain the maximum rapidity of flow in the fingers as well as a rapid flow in the legs, until some slow process of acclimatization had been achieved. In the cold the normal change was seen though in the cold room the maximal values obtained did not reach the accepted dilatation values. The value on the first day of cold was markedly higher than those on subsequent days.

Calibre of veins. Photographs of veins with infra-red were made either daily or at frequent intervals in the experiments. The camera was fixed and the position of the subject maintained constant so that exact comparisons could be made. There was some evidence that during acclimatization to warmth the veins became slowly larger and more numerous even though the environmental temperature remained the same. The effects of cooling were more definite; full constriction of the veins was not attained except after exposure to the mild cold for 2 or more days. Photographs on subject C in experiment 2 are shown in figure 7. Attempts were made to confirm these observations by measurements of the forearms in a water plethysmograph in experiments 3 and 4. A definite correlation between the volume of the limb and the dilatation of the veins as indicated by photographs appeared to be present. The volume changes were however small relative to the accuracy of measurement. The mean value for the forearms of the two subjects in experiment 4 was 1881 ml. in the cool, 1885 for the first two and 1898 for the last two days of warmth; on the succeeding 2 cool days the mean was 1876. In experiment 3 the mean for the two subjects was 1314 ml. throughout the period of warmth;

the mean for the cold days was 1275. On the first two days of cold the mean was 1290, on the 3rd 1226, and on the last two days 1280.

Discussion. When the temperature of a room is raised as in these experiments there is a considerable increase in surface temperature, but only a small increase in the deep body temperature. Heat balance is maintained but at a high level for *mean body temperature*, a change which in itself increases heat production. As acclimatization is attained this increased heat production is gradually reduced. With this there is a partial disappearance of the slight rise in the deep body temperature, but surface temperatures remains high and there is little reduction in mean

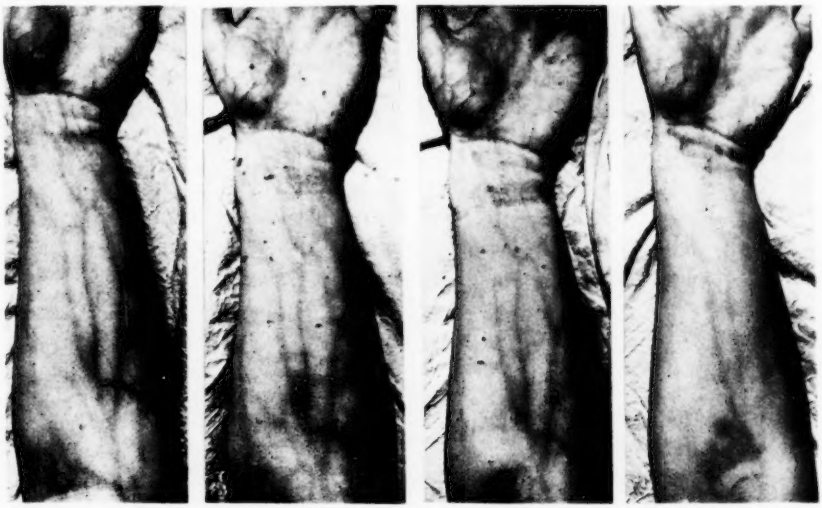

Fig. 7. Infra-red photographs of the arm veins in subject C in experiment 2 (summer). From left to right are shown the first and last days of warmth and the first and last days of cold.

body temperature. The subject becomes more comfortable but this seems to depend mainly on more efficient mechanisms of heat loss than on reduced heat production. The adjustments are attained by modifications of the partition of heat loss in which a considerable increase in blood volume appears to play some part. When the room temperature is lowered the reverse changes take place but again the adjustments cannot be attained immediately. During the initial exposure to cold full constriction of vessels in the fingers and of superficial veins is not readily attained. Blood volume appears to be rapidly reduced, in the sense that blood is removed from the circulation to some area where it is not readily available to carbon monoxide. However the red cells do not for the most

part seem to have been destroyed, for sudden returns towards the volumes observed in the warmth develop; these increases do not attain the levels found in the warmth, nor do they persist. Presumably the blood volume is slowly and steadily reduced, while the circulating volume shows phasic variations as more or less blood is side-tracked into reservoirs.

The whole circulation also shows considerable changes as indeed must be the case, for the alterations in blood volume imply variations of 20, 30 or even 40 per cent in the vascular bed. In the warmth the flow in the fingers steadily increases with acclimatization and the peripheral veins become larger. This suggests that the most peripheral parts of the limbs play an increasing rôle in heat loss (probably both from radiation and evaporation). If this blood can be returned slowly along superficial veins economical cooling should be attained, but considerable quantities of blood would be required to fill such a system. Such a tentative hypothesis offers an explanation of a slow vascular adjustment limited in speed by changes in blood volume. Circulatory changes and those in blood volume proceed at approximately the same rate and both show phasic changes in exposure to cold after warmth. Such phasic changes are the invariable rule in finger flow (see fig. 6), and not uncommon in the blood pressures and pulse rates (figs. 2 and 3).

The other changes in the circulation can mostly be explained in line with this hypothesis. The initial response to heat is a peripheral dilatation wide-spread over the skin accompanied presumably by some central vasoconstriction. The increased blood flow in the skin combined with an increased metabolism gives a high cardiac output. The vascular bed is enlarged and fluid is transferred from the tissues to the blood, but only slowly as plasma proteins have to be added for this transfer to attain its full development. The effective peripheral resistance is lowered but not to the extent that the cardiac output is raised so that the blood pressure level rises.

With acclimatization to heat and increase in blood volume the partition of heat loss is more efficient and blood flow to the skin as a whole can be reduced. Metabolism is also reduced (see Burton et al., 1940). Cardiac output can return to a normal level even while blood flow in the fingers is increasing, and blood pressure falls. When the subject stands a peripheral dilatation exaggerates the interference with venous return until the blood volume has been increased. Later the increased blood volume, which has served to relieve the strain of a maintained peripheral dilatation to heat, serves also to relieve the strain imposed by venous stasis. In the process of these adaptations the oxygen supply to the bone-marrow is inadequate either through involvement of the nutrient vessels in vasoconstriction or through dilution of the blood, and formation of new cells is therefore stimulated.

On exposure to cold following warmth full constriction is retarded until the blood volume has been reduced. Central arteriolar vasodilatation is necessary to balance the effect of constriction in the skin vessels (table 1, 27 and 28) so that little increase in peripheral resistance develops. Metabolism falls and cardiac output is reduced. The fall in cardiac output causes a fall of blood pressure, for the enlarged blood volume would interfere with compensatory central constriction. One can more easily explain the blood pressure changes both to warmth and cold if one assumes that venous filling of the heart under these conditions is exerting a reflex influence on blood pressure control. At first in the cold the venous return is well maintained on standing, the stroke volume is high and the pulse slow. As blood volume is reduced, these characteristics disappear.

The fact that a sequence of warmth followed by cold causes a rise in the blood pressure levels, particularly diastolic pressure, implies that the results are not simply reversible. Certainly during the reduction in blood volume there is an increase in hematocrit and hemoglobin percentage and blood viscosity is no doubt increased. In many cases plasma protein concentration may be greater (subject F, expt. 3) and this should raise not only blood viscosity but colloid osmotic pressures. However the blood pressure changes were very similar in subjects E and F (fig. 3), though F alone showed any great increase in plasma protein concentration and colloid osmotic pressure; the observed difference in colloid osmotic pressure between the two subjects did not seem to modify other reactions. It has been shown that in the response to cold the insensible loss of water through the skin may be very low. Such low levels have been associated with low levels of mean arterial pressure (and presumably of capillary pressure) relative to the colloid osmotic pressure. Here again no marked difference was seen between subjects E and F in spite of the differences in colloid osmotic pressure. Even the present tentative analysis fails to explain these factors.

Reference to pulse wave velocities and to the distensibilities of vessels calculated from them in table 1 shows that the distensibilities of the more peripheral vessels are greater in the warmth. This presumably implies relaxation of their muscular coats. On the contrary the distensibility of the more central vessels is greater in the cold, and particularly so after a period of warmth. One may assume that these central vessels dilate and act as a reservoir, and help to accommodate excess blood. The changes in central pulse wave velocities are not so great as those seen in acute experiments with baths (Bazett et al., 1937) but are in the same direction. During standing PWV I is decreased, suggesting an increased reservoir effect of the arch of the aorta under the conditions of higher blood pressure in this posture. This decreased velocity (and increased reservoir action) is more marked in the warmth, when the blood volume is high than in the

cold where the blood volume is lower. The same differences in pulse wave velocity were noted previously as an effect of climate (Bazett et al., 1935).

Little reference has been made to experiments where warmth was interrupted by a brief period of cold for these experiments need much further work. However it may be stated briefly that such an exposure to cold modified the acclimatization to warmth as far as the circulation is concerned; much of the process of adaptation had to be repeated but it progressed much more rapidly. The metabolism instead of decreasing in the warmth appeared to increase. In the normal exposure of an unacclimatized person to heat, all subjects have complained of headache for one day (about the 3rd to 5th day). This has been accompanied by loss of appetite. It has only been in evidence once in summer experiments and never in a second period of warming.

If a subject is not acclimatized and is exposed to heat, signs of unfitness are found in a mental inaccuracy of an anoxemic type which is rarely recognized by the subject. Also if he stands much, frank edema of the feet and ankles has sometimes been seen; this disappears with acclimatization. Serum protein concentration has been normal at the time of the edema.

The hypotheses advanced must be regarded as entirely tentative, and as only applicable even tentatively to situations when thermal conditions are maintained relatively constant for days. The cardiovascular responses are determined by the previous thermal history of the individual and at present nothing is known of the effect of fluctuating temperatures. The lack of correlation between the thermal conditions of the moment and cardiac output while standing reported by Scott (1936) was found during a year with very irregular thermal conditions; the absence of correlation in these observations need not be considered as conflicting with the results here reported.

SUMMARY

1. Observations on pulse rates, blood pressures, pulse wave velocities, and cardiac outputs calculated from such values, as well as determined by acetylene, are reported in six subjects exposed to moderately warm or cool rooms continuously for several days. The total observations involved 87 subject-days. The reactions of the individuals are found to be modified by their previous thermal history. Two subjects kept in the same room usually reacted similarly. Greater differences were seen between two experiments than between two subjects in any single experiment.

2. Cardiac outputs with the subjects lying down are increased at first in the warmth and then subside to normal or high normal basal values. In the cold they are at first reduced to subnormal levels and return to normal levels.

3. Cardiac outputs with the subject standing show marked differences according to the thermal history of the subjects. After adaptation to cool conditions the cardiac output with the subject standing is much below that observed with the subject lying down. In the early stages of warmth this reduction is exaggerated. In later stages of adaptation to warmth there is on the other hand little or no such reduction in cardiac output. A series of 42 observations of cardiac output in the standing posture made on 2 subjects within a period of 2 weeks is subjected to statistical analysis to demonstrate this point.

4. In any given individual these effects of adaptation to warmth may be absent if the subject is dehydrated. Dehydration may exist even though the subject may be free of symptoms at the time of examination.

5. The differences between the pulse rates while lying and standing are exaggerated in the early stages of exposure to warmth and are reduced later. If exposure to cold follows warmth they are at first reduced still further, and then later increased.

6. The efforts of prolonged but relatively mild changes in environmental temperatures on the blood pressures are an initial rise and later fall in the warmth and an initial fall and later rise in the cold. This sequence leads to pressure levels which considerably exceed those control values which precede it. The possible causes are discussed.

7. Marked differences may be seen in the same subject examined both in the morning and in the afternoon in his reaction to standing. As judged by increases in pulse rate and change in blood pressure on standing, or by cardiac output while standing, an individual may appear to become daily less fit in the mornings on the same series of days during which he appears to be becoming more fit in the afternoons. Fitness tests are complicated by the changes here reported.

8. The maximal rate of blood flow attained in the fingers steadily increased in a warm room with acclimatization of the subject and reached values much above those commonly found. Warming of the legs decreased, rather than increased, these flows and the decrease was greater, the less the subject was acclimatized. The decrease was also greater if the subject showed signs of dehydration. These changes are attributed to a blood volume relatively inadequate for the demands of the circulation in the early period of exposure to warmth. On exposure to cold several days were required to attain maximal constriction in the fingers. This is attributed to a large blood volume.

9. The veins of the forearm examined by infra-red photographs showed a slight, though somewhat indefinite, gradual increase in size during acclimatization to warmth. They also showed an incapacity to constrict maximally to a mild stimulus of cold in the first few days of such exposure.

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THE HEAT REGULATION OF SMALL LABORATORY ANIMALS AT VARIOUS ENVIRONMENTAL TEMPERATURES

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The pronounced effect of environmental temperature on the heat production of small warmblooded animals is well known. The general practice of controlling external temperature during metabolic determinations recognizes this factor and the condition maintained is normally that temperature which experimentation has indicated as productive of a minimum metabolism for a given species. Investigators agree that the critical temperatures for the usual laboratory strains of rats, mice, and guinea pigs lie within the range from 27° to 31°C., and common practice has favored the use of conditions between 28° and 30° (Benedict, 1938).

It is much more difficult, however, to assemble from the literature reliable information on the effect of temperatures below the area of thermal neutrality. The increasing use of temperature extremes in conjunction with endocrine research, fever therapy, tumor growth, and related problems has given a new usefulness to such information for many studies in which the smaller animals are suitable reagents.

Recent studies on the biophysics of human heat loss (Burton and Bazett, 1936; Hardy and DuBois, 1937; Winslow, Herrington, and Gagge, 1937; Gagge, 1937) have made possible a more precise definition of the biologically effective environmental temperature and emphasized the necessity for controlling such neglected factors as radiation temperature and air movement in animal as well as human experiments. These studies also suggest that a better understanding of temperature regulation in animals may be gained through the inclusion, in studies of thermal effects, of data on surface temperature and heat conductance in addition to the usual data on heat production.

The present study of the metabolic response of the albino rat, the mouse, and the guinea pig at successive small temperature intervals between 13° and 35°C. has, therefore, been organized with two principal aims; first, the provision of standards of heat production of general utility for laboratories using these animals under various temperature conditions, and second, the determination of the surface and internal temperatures, and thermal conductances needed in a description of the adjustment from the standpoint of heat loss.

EXPERIMENTAL CONDITIONS. The metabolic response of a small homoiothermic animal to a given temperature is modified by the standard temperature at which the animal has previously lived (Schwabe, Emery, and Griffith, 1938; Gelineo, 1935). This adaptation is slow and usually involves a change in the protective coat and fat insulation (Hoesslin, 1888), and possibly an endocrine change which alters the functional response to a given change in average body temperature. All animals used for these tests were males and had lived in an environment maintained within the limits of 23° to 25°C. Prior to the metabolism trial they were transferred to the test temperature for fasting periods of 12, 18 and 24 hours for mice, rats and guinea pigs, respectively. This routine allows adequate short term thermal and nutritional adaptation and standardizes the most immediate stimulation of activity which accompanies a shift in environmental temperature.

At the conclusion of the preliminary period groups of animals of a single species were placed in *individual* compartments of a group metabolism chamber and after one hour a standard metabolism period of 6 hours was run, from 10 a.m. to 4 p.m. Animals were then removed to the standard environment of 24° and lived there until the time of the next test, which normally was not less than one week.

Animal stocks were from a single source,[1] and all were mature. Weight increases occurred during the study—in the mice from 20 to 31 grams, in the rats from 310 to 377 grams, and in the guinea pig from 389 to 757 grams. These weights correspond to ages of 120 to 350, 300 to 500, and 360 to 700 days for mice, rats, and guinea pigs, respectively. The mice and rats were fed a stock preparation (Tioga laboratory ration), and guinea pigs on oats and lettuce.

The conditions which are desirable for comparable measurements of metabolism are well known (Benedict, 1938). With two exceptions the conditions met these accepted requirements. By design the experiments were not to be made exclusively at the critical temperature, but this condition was observed in principle by maintaining a constant home cage temperature and providing adequate adaptation to the test temperature. In addition, the criterion of activity could not be met, since modification in activity is unquestionably a significant part of temperature adaptation in the animals studied. In making such a statement we accept the viewpoint which considers that immediate chemical regulation (as distinct from long-term adjustment) is due essentially to increased muscular activity. Under such a view, measurements below the critical point are not representative if made only in quiet periods. Since no measurements under any conditions are ever strictly basal, there appear to be no serious objections and many advantages (particularly with small animals) in

[1] Animal Breeding and Laboratory Institute, New York.

accepting activity as normalized by populations and long periods as a condition of the standard metabolism, both within and without the zone of thermal neutrality (Barbour and Trace, 1937).

All results have been obtained on trial runs in which the total metabolism of individually caged groups of 6 guinea pigs, 10 rats, and 50 mice, respectively, was determined.

Determination of metabolism. The Haldane gravimetric method proved most convenient for our purposes and equipment.

A cylindrical chamber 36 inches in length and 18 inches in diameter was used. The cylinder was constructed of aluminum struts fastened to brass rings which were flanged and machine surfaced to receive, on one end, a soldered copper cap, and on the opposite end a pressure-locked removable copper head. The surface was covered with heavy copper foil soldered at the ends of the rings. A 20 kilogram Sauter balance (50 mgm. sensitivity) was employed for weight determinations.

The experimental animals were individually caged in small hemispherical wire mesh containers. These were supported by an aluminum rack which slid into the chamber. The diameter of the wire containers was determined on the basis of the average adult length of each species. In group metabolism as in individual measurements, every precaution is necessary to insure that the radiation temperature of the environment does not vary significantly from the air temperature. The use of chamber walls of high thermal conductivity and control of air temperature both within and without the chamber accomplished this in part. In addition it was necessary to space animals so that no important percentage of the solid angle subtended by their surface was obstructed by the surfaces of other animals. Experiments with mice provided the conditions of greatest crowding in this study. Computations of this shielding effect with cages 6.4 cm. in diameter and an average animal diameter of 1.5 cm., assuming orientation as parallel cylinders between animals, indicated that less than 15 per cent of the total solid angle might be obstructed. The use of 20-mesh caging reduced this effect to about 9 per cent. The temperature of the mouse's surface varied from 9° to 4° above air over the experimental temperature range. Under these conditions the maximal radiant effect on operative temperature (recorded air temperature) decreases from approximately 0.4° at 15°C. to 0.2° at 29°C., the average effect (considering orientation factors) being much less and quite unimportant. The use of 20-mesh caging served to standardize air movement inside the containers within the range from 12 to 20 feet per minute, which provided adequate ventilation without exposing the animals to forced air movement. It should be emphasized that a standard rate of ventilation in volumetric terms does not adequately define this factor. As a result the majority of reported experiments have a possible constant error range of several degrees in operative temperature. Ventilation rates must be specified

in anemometric as well as volumetric terms (Winslow, Gagge, and Herrington, 1939).

The complete apparatus was set up in an air-conditioned room within which the temperature could be controlled to ±0.25°. The air intake for the chamber was located in this room, an arrangement which permitted the apparatus to be maintained at any desired temperature without complicating the chamber air circuit with heating or cooling devices. A ventilation rate of 1200 liters per hour was sufficient to prevent any

TABLE 1

Metabolism of albino rats at various temperatures

T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER	T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER	T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER
	grams				*grams*				*grams*		
34.0	366	1178	66	28.9	350	705	18	23.9	356	766	30
33.2	345	884	65	28.9	351	744	11	23.6	351	801	29
32.2	316	918	20	28.4	376	588	49	23.5	374	814	28
32.2	351	873	21	28.4	376	696	56	23.3	321	856	4
31.9	353	852	24	28.2	357	699	41	23.1	373	820	57
31.9	340	843	22	28.1	371	660	40	22.6	330	808	2
31.2	355	863	52	27.8	367	655	43	21.9	377	916	64
30.8	345	848	23	27.8	335	744	13	21.1	338	993	31
30.7	350	798	54	27.7	335	703	17	21.1	351	872	7
30.6	356	511	19	27.2	358	713	39	20.8	357	1001	33
30.5	357	859	45	26.9	359	679	26	19.7	362	1043	37
30.5	350	843	46	26.8	348	752	27	19.2	371	1042	59
30.0	372	744	55	26.3	370	717	38	18.6	348	1082	36
29.8	368	809	48	25.7	367	694	25	18.2	347	1073	34
29.6	365	842	53	25.0	343	826	58	18.1	375	1017	32
29.4	353	783	42	25.0	350	760	10	18.1	371	1085	35
29.4	353	742	47	24.4	312	728	8	18.1	366	1130	60
29.4	373	722	51	24.4	310	852	1	15.4	358	1243	50
29.4	322	655	16	24.4	335	837	9	15.3	346	1254	62
29.3	351	698	44	23.9	309	921	3	13.6	376	1304	61
29.2	317	813	12	23.9	352	815	6	13.6	345	1410	63
29.1	364	662	14	23.9	319	803	5				

T_A = Air temperature in degrees Centigrade.

significant deviation of the chamber temperature from the room temperature. Groups were placed in the metabolism chamber for one hour prior to the determination of the initial weights of the chamber and the CO_2 absorber, and the final weights taken after a period of 6 hours. Manometric tests under slight negative pressure were made prior to each run as a control on the tightness of the air circuit. CO_2 absorbent bottles were charged daily to insure a wide margin of safety, and H_2SO_4 renewed after a standard increase in volume.

Computation of metabolism. The independent weights of the CO_2

absorber and the chamber permitted the determination of the CO_2 directly and O_2 consumption by difference. R.Q. values for 65 technically acceptable rat periods, 63 mouse periods, and 88 guinea pig periods had mean values of 0.741 ± 0.042, 0.739 ± 0.031, and 0.765 ± 0.035, in the same order. Plots of R.Q. against test temperatures show a random distribution. Mean R.Q. values are similar to those found in the other studies (Horst, Mendel and Benedict, 1930; Swift and Forbes, 1939). Absolute variability is somewhat greater than would be expected in individual experiments

TABLE 2

Metabolism of mice at various air temperatures

T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER	T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER	T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER
	grams				*grams*				*grams*		
35.3	23.9	1009	8	28.3	28.0	752	69	22.5	28.8	951	29
35.2	27.1	803	64	28.2	23.0	928	3	22.1	28.2	862	31
35.0	19.6	1159	62	28.0	28.1	793	68	22.0	27.8	1010	32
35.0	31.0	870	54	27.7	27.3	800	24	21.7	29.3	957	27
34.8	25.0	1013	61	27.6	31.1	742	56	21.4	31.0	1169	49
34.6	30.6	724	53	26.9	28.1	835	37	20.8	27.4	1228	22
33.9	29.3	894	52	26.6	31.1	973	57	20.8	26.2	1088	23
33.9	23.8	911	6	26.4	28.2	922	35	20.0	29.1	1037	26
33.7	24.1	930	60	26.3	30.6	927	41	18.9	30.3	1360	48
32.8	27.5	583	66	25.8	29.3	856	36	18.1	28.8	1276	45
30.6	27.7	655	67	25.6	27.4	961	21	18.0	26.0	1170	17
30.2	28.4	763	25	25.3	29.0	945	47	17.7	26.8	1206	16
30.1	26.8	883	65	25.2	28.0	794	39	16.8	26.1	1171	18
29.9	21.5	879	59	24.9	28.1	953	43	16.6	26.3	1255	15
29.8	31.8	814	55	24.7	26.2	1013	44	16.0	25.1	1307	13
28.9	24.0	832	1	23.4	28.8	1075	33	15.5	26.2	1365	14
28.8	23.3	808	5	23.3	28.4	906	38	14.6	29.1	1741	50
28.7	28.1	733	70	23.3	28.5	1013	30	14.1	25.6	1492	10
28.6	28.1	680	34	23.0	27.9	940	19	14.0	24.1	1518	9
28.4	24.0	819	4	22.9	27.0	846	20	13.9	24.9	1371	12
28.3	23.7	808	2	22.9	28.8	928	28				

T_A = Air temperature in degrees Centigrade.

and is due to the difficulty in realizing the full sensitivity of the balance in weighing a chamber occupied by groups of animals which are never completely quiet. This difficulty does not apply to the CO_2 determination nor does it affect significantly the reliability of the mean R.Q. in a long series, as can be demonstrated in these data by comparison of mean R.Q.'s for odd and even numbered experiments.

In computing the heat production the CO_2 value has been used in conjunction with the mean R.Q. value for the entire series on a given species. Test temperatures, the metabolism per unit of surface and the mean animal weights for each experiment are given in tables 1, 2 and 3.

RESULTS. In figures 1, 2 and 3 the results of 64, 63 and 88 group experiments on rats, mice and guinea pigs, respectively, have been plotted against test temperatures from 13° to 35°C. Heat production has been computed in terms of Calories per square meter per 24 hours using the

TABLE 3

Metabolism of guinea pigs at various air temperatures

T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER	T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER	T_A	AVERAGE WEIGHT	KGM.-CAL. PER M² PER 24 HOURS	EXPERIMENT NUMBER
	grams				*grams*				*grams*		
35.3	754	716	69	27.9	643	720	94	21.1	603	894	31
34.7	626	557	72	27.8	577	678	2	21.0	577	815	30
34.7	640	600	73	27.8	548	677	3	20.9	698	898	47
34.6	660	885	70	27.8	664	739	99	20.7	566	812	29
34.6	705	719	68	27.7	661	777	97	20.3	580	839	33
34.3	669	694	71	27.6	637	700	76	20.2	701	988	52
33.6	589	620	84	27.2	686	693	101	20.0	731	913	51
32.8	543	701	5	26.8	692	753	43	19.7	584	968	28
32.5	649	673	83	26.7	639	713	44	18.1	551	991	16
32.2	644	579	77	26.3	700	766	56	17.6	722	1005	62
32.0	757	687	82	26.1	706	725	54	17.5	550	935	17
31.9	527	676	4	25.7	681	893	48	16.7	540	917	21
31.4	567	638	27	25.4	779	752	59	16.7	537	999	14
31.2	656	644	81	25.3	737	801	53	16.4	707	971	65
31.2	639	594	79	24.8	812	784	57	16.4	544	959	19
30.9	606	607	87	24.2	739	789	55	16.4	556	983	15
30.9	564	621	88	23.7	641	787	93	16.4	535	993	20
30.7	562	586	26	23.2	659	794	42	16.3	555	1023	22
30.3	613	561	78	23.1	565	891	24	15.8	553	1071	18
30.3	610	592	74	23.1	699	797	50	15.4	771	1065	61
29.8	680	601	92	23.1	600	835	34	15.1	754	1050	67
29.6	548	703	91	23.0	625	855	38	14.0	539	1057	12
29.4	646	583	86	22.7	666	799	39	13.6	508	1057	8
29.4	596	672	85	22.7	682	905	45	13.5	489	1104	9
29.3	657	628	80	22.6	705	804	49	13.5	656	1157	66
29.1	652	601	75	22.6	652	833	36	13.3	530	1085	10
28.8	690	670	100	22.5	604	810	40	13.3	561	1347	13
28.8	681	605	98	22.4	632	742	37	13.1	513	1071	11
28.5	691	756	102	22.2	634	852	35				
28.2	587	631	90	21.6	623	877	41				

T_A = Air temperature in degrees Centigrade.

formula $9.0 \times W^{2/3}$ for rats and guinea pigs, and $9.1 \times W^{2/3}$ for mice (Benedict, 1938). The mean individual weight for a given group has been employed in computing the area relationship. In tables 1, 2 and 3 the exact values for the plotted points are tabulated.

The marked effect of temperature on the heat production of all three

species and the essentially linear increase below the approximate area of thermal neutrality is apparent. These increases in heat production with decreasing environmental temperature may be expressed by the following equations:

(1) Cal./M²/24 hours = 1879 − 43.2 T (°C.) (Rats—below 28.9°)
Standard error of estimate ± 23.9

(2) Cal./M²/24 hours = 2093 − 46.3 T (°C.) (Mice—below 30.6°)
Standard error of estimate ± 35.4

(3) Cal./M²/24 hours = 1426 − 26.1 T (°C.) (Guinea pigs—below 30.0°)
Standard error of estimate ± 46.9

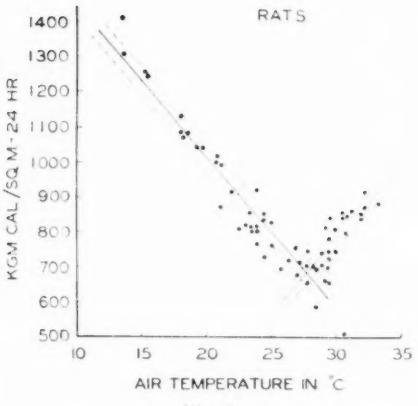

Fig. 1

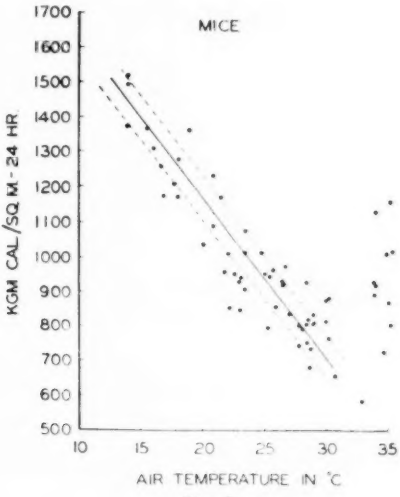

Fig. 2

Fig. 1. Standard metabolism of albino rats at various temperatures. Each point represents the mean of 10 animals.

Fig. 2. Standard metabolism of mice at various temperatures. Each point represents the mean of 50 animals.

The greater variability of results and the short range available above the critical temperature areas prevented the accumulation of equally reliable data in the hot region. In only one instance have the data justified a fit in this range.

(4) Cal./M²/24 hours = −503 + 42.8 T (°C.) (Rats—above 29.2°)
Standard error of estimate ± 15.5

These data have been used to determine the temperature intervals of minimal metabolism for the three species by smoothing both the test temperature series and heat production values with a moving average of

three. In table 4 the averages of the smoothed metabolism values for each degree of test temperature between 27° and 33° are given.

Inspection of this table indicates minimal values of heat production for rats and guinea pigs between 28.0 to 28.9° and 30.0 to 30.9° respectively. The minimal point for mice is not clearly indicated due to the absence of data in the 31° interval. It seems certain, however, that the critical point is above 30° and under 33°, and it is likely that the 31.0 to 31.9° interval is

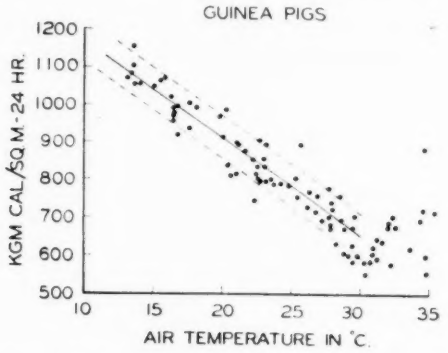

Fig. 3. Standard metabolism of guinea pigs at various temperatures. Each point represents the mean of 6 animals.

TABLE 4

Heat production in Cal. per M² per 24 hours between 27° and 33°C.

	TEMPERATURE, °C.											
	27.0-27.9		28.0-28.9		29.0-29.9		30.0-30.9		31.0-31.9		32.0-32.9		33.0-33.9	
Rats	701+ 9†	4*	679+10	6	746+36	9	772+44	6	853+ 3	3	887+ 6	2		
Mice	785+ 7	2	794+30	9	851+ 9	2	759+61	3			723+ 0	1	860+52	2
Guinea pigs	642+41	7	661+18	4	635+ 7	6	593+10	5	640+20	4	658+13	4		

* Number of experiments in each interval.

† Average deviation of mean from smoothed values.

a best estimate of its location. With the above reservation in mind, it may be concluded that 28.5°, 30.5°, and 31.5° are temperature points which are acceptable as conditions of thermal neutrality for rats, guinea pigs, and mice, when normal activity is allowed. Under these conditions metabolisms of the order of 680, 590, and 740 Cal./M²/24 hours, in this same species order, are found when surface area is computed as described earlier. The critical temperatures indicated by these data are in rough agreement with those cited by Benedict (1938) for rats and mice; the

critical temperature for guinea pigs is, however, definitely higher in this study.

Physical factors in temperature regulation. The most conspicuous point bearing on the comparative temperature regulation of the three species (see figs. 1, 2 and 3) is obtained from the slopes of the lines on the cold side of the zone of thermal neutrality. For a standard decrease of 1°, in environmental temperature, the metabolic increases in Calories per square meter per 24 hours are 46.3, 43.2 and 26.1, for mice, rats and guinea pigs, respectively. From a biophysical standpoint the relative Calorie cost of maintaining a constant internal body temperature for animals of similar pattern, efficient internal circulation, and comparable peripheral insulation, should be proportional to the ratio of surface area to body volume, or its

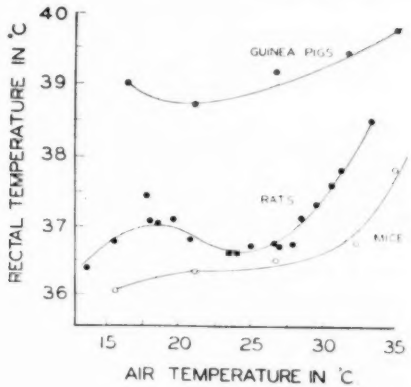

Fig. 4. Rectal temperatures of guinea pigs, rats, and mice. Each point represents the mean of 10 animals.

approximate, body weight. In the present case, the mean weights of 27.1, 351 and 629 grams for mice, rats and guinea pigs, respectively, indicate, on the basis of surface area formulae previously cited, 0.299, 0.129 and 0.105 sq. m. per kilogram of body weight. If the increase in heat production per unit of temperature change were solely a function of the relatively greater surface of the smaller species, the observed increase for each species for 1° of environmental cooling should be proportional to its surface-weight ratio. Actually, we find the relative surface-weight ratios for guinea pig, rat, and mouse, to be 1, 1.23 and 2.81; the ratios of the heat increases (in the same order) 1, 1.66 and 1.77. In a rough sense the conception that the Calorie cost of maintaining a constant body temperature increases with decreasing size is borne out by the order of these two sets of ratios.

In figure 4 are presented the mean rectal temperatures for the three spe-

cies over a wide range of environmental temperatures. Groups of 10 of each species were measured at each temperature. Variations from ±0.4° to ±1.6° around mean points occurred, and the mice were least constant. Care was taken to insert thermocouples deeply. Depths of 8 centimeters for the guinea pig, 5 for the rat, and 2 for the mouse were marked on the couples, and it was generally possible to insert them to this depth. Gradients of 1.5° to 2° between full and half insertion were common, particularly at the colder air temperatures. These measurements were taken under fasting conditions and after the same periods of adaptation used in the regular metabolism series. It is at once apparent that the guinea pig maintains a higher body temperature in the range from 15° to 32° than

TABLE 5

Conductance values at 26.7°C.

	GUINEA PIGS	RATS	MICE
Surface temperature	31.1° ± 1.2°†	30.8° ± 1.6°	31.2° ± 1.9°
Rectal temperature	39.2° ± 0.7°	36.7° ± 0.9°	36.5° ± 1.3°
Metabolism*	736 ±47	727 ±24	859 ±35
Metabolism-evaporation	626	618	720
Temperature difference, °C.			
Surface-air	4.4°	4.1°	4.5°
Rectal-surface	8.1°	5.9°	5.3°
Rectal-air	12.5°	10.0°	9.8°
Conductance kgm.-Cal. per M^2 per 24 hours—°C.			
Surface-air	142.3	150.7	162.2
Rectal-surface	90.9	123.2	162.1
Rectal-air	55.3	67.9	81.0

* Metabolism and evaporation in kgm.-Cal. per M^2 per 24 hours.
† Standard deviation.

either rat or mouse, and with little mean variation over the range. The temperature of the rat does not approximate that of the guinea pig until an environmental temperature of about 35° is reached, and although the data do not go to this point, it is certain that the internal temperature of the mouse is considerably below that of the guinea pig at air temperatures even above 35°. At temperatures from 15° to 30° the rat, with a surface-weight ratio only 20 per cent greater than that of the guinea pig, must generate about 65 per cent more heat per unit of surface for each degree fall in air temperature in order to maintain a rectal temperature which is, on the average, 2° below that of the guinea pig.

Such species differences in effective insulation may be quantified in the following manner. In table 5 are given the mean surface and rectal tem-

peratures, metabolisms, and thermal conductances, for a group of fasting guinea pigs, rats, and mice, at an air temperature of 26.7°C. The metabolism at this air temperature has been taken from figures 1, 2 and 3. The heat conductances for the three species have been computed in a manner similarly employed in human studies (Burton and Bazett, 1936; Winslow, Herrington, and Gagge, 1937). In these computations the several conductances are computed from the following formulae:

$$\text{Internal Conductance, } K_{T_R-T_{Sur}} = \frac{M}{T_R - T_{Sur}}, \text{ (Rectal-surface),}$$

$$\text{External Conductance, } K_{T_{Sur}-T_A} = \frac{M - E}{T_{Sur} - T_A}, \text{ (Surface-air),}$$

$$\text{Total Conductance, } K_{T_R-T_A} = \frac{M}{(T_R - T_A) + \frac{E}{K_{T_{Sur}-T_A}}}, \text{ (Rectal-air).}$$

where M and E represent metabolism and evaporative loss[2] expressed in Calories per square meter per 24 hours, and temperatures are in degrees Centigrade. T is the general symbol for temperature, R, Sur, and A, refer to rectum, surface and air, respectively.

The external conductance, $K_{T_{Sur}-T_A}$, is a function of body form, surface character, and posture, and is a relatively stable physical constant which describes the heat loss by radiation and convection per degree Centigrade difference between surface and air when the conditions of air movement in the environment or as induced by the activity of the animal are held constant. In the present case we find little difference in the values for $K_{T_{Sur}-T_A}$ for the three species, and it is probable that the small variation noted reflects the relative degree of activity,[3] the mouse being most and the guinea pig least active.

Both $K_{T_R-T_{Sur}}$ and $K_{T_R-T_A}$, however, are approximate indices of the effective insulation of the peripheral tissues and coat, the former more specifically so. An inspection of either of these indices shows that there is a progressive decrease in the effectiveness of the peripheral insulation from guinea pig to mouse. This conclusion, expressed in conductance terms, is also readily deducible from the fact that although rat and guinea pig have approximately equal heat production per unit surface at 26.7°, the rat's rectal temperature is 2° lower, and likewise a 15 per cent greater heat

[2] Measurements of evaporative loss for the three species at temperatures below 29° have varied between 12 and 21 per cent of the metabolism. For these computations it is entirely practical to assume a standard value of 15 per cent.

[3] Activity has the same effect as an increase in air movement, namely, an increase in the convection constant.

production for the mouse is only barely sufficient to maintain its internal temperature at a point 2° to 2.3° below that of the guinea pig.

In figure 5 are plotted the overall conductances from rectal to air temperature computed on the basis of a 15 per cent insensible perspiration loss. Data for the three species raised at 23° to 25° and, for comparison, data on "hot" rats (31°–32°), and "cold" rats (17°–19°), and clothed human subjects, are included. The temperatures indicated for the rats are the temperatures maintained in the home cages of these special groups.

It is interesting to note that normally clothed human subjects have a conductance value approximating that of the mouse. From the standpoint of heat regulation, however, this equivalent conductance is maintained in the human being *without* any increase in metabolism and the

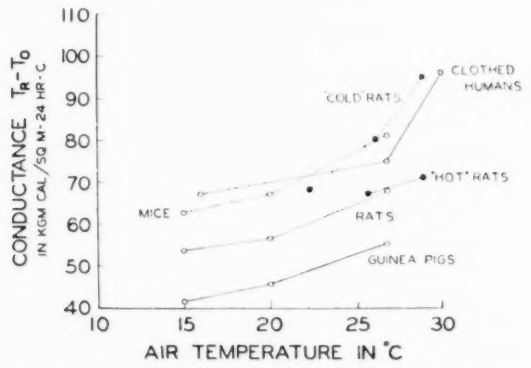

Fig. 5. Overall thermal conductance, rectum to air, for various groups of animals and clothed humans. T_O is defined as $T_A - \frac{E}{K_{T_{SUR}} - T_A}$.

reasonable conclusion is that both effective vasoconstriction and the ability to tolerate a large cooled peripheral mass are the elements which give the larger human body its temperature protection.

Another point of interest is the fact that the conductance of "cold" rats (notably lean and small as a result of retarded growth) is greater than that of the "hot" or normal rats. "Hot" rats have a low conductance, but it appears to change less rapidly than does that of the "cold" rats, presumably because the former have had little adaptive experience with cold temperatures.

Although conductance decreases for all three species with decreasing environmental temperature, this does not indicate the existence of a conspicuous physical regulation through control of peripheral circulation, but

more probably an increase in the depth of the temperature gradient. An indication of this increase in the internal gradient has been seen in measurements of the rectal temperature of mice and guinea pigs for different depths of insertion at normal and low temperatures. This progressive thermal failure must occur since, if we take the guinea pig as an example, we find that a drop of 1° in air temperature would require an increase in the rate of heat production of 142 Calories per square meter per 24 hours to maintain a constant skin temperature, while the increase in metabolism for 1° is only at a rate of about 26 Calories per square meter per 24 hours. This illustration emphasizes the fact that the guinea pig achieves a new equilibrium after a fall in environmental temperature at the expense of an indefinitely lower average body temperature despite increased heat production. When a drop of 1° is produced in the environment, the animal does not continue to lose an extra 142 Calories. What does occur is a progressive drop in skin temperature which, at some lower value, reaches a new equilibrium with the increased metabolism.

An assessment of the approximate value of this decrease in average body temperature can be made. From experiments in our laboratory with shaved and normal rats, we have found that the conductance value obtained for the rat's coat of fur is of the order of 225 Calories per square meter per 24 hours per degree Centigrade. Rubner (1895) has reported a conductivity value for rabbit fur of 90 Calories per square meter per 24 hours per degree Centigrade per centimeter. If we assume 0.5 cm. as the depth of the guinea pig's protective coat, this would yield a conductance value of 180 Calories per square meter per 24 hours per degree Centigrade. We will use this latter value, since it is quite certain that the guinea pig has a degree of peripheral insulation greater than that of the rat and less than the rabbit.

From table 2 we obtain for 26.7° for the guinea pig, a total conductance of 90.9 Calories from rectal to surface temperature. This conductance contains both the peripheral factor and the rectal-true skin factor. By the use of conductance reciprocals (resistivities) we can estimate the skin temperature at the outer tissue (not fur) surface.

$$(1)\quad \frac{1}{K_{T_R-T_{Sur}}} - \frac{1}{K_{T_S-T_{Sur}}} = \frac{1}{K_{T_R-T_S}}, \qquad (2)\ K_{T_R-T_S} = \frac{M}{T_R-T_S},$$

$$\frac{1}{90.9} - \frac{1}{180.0} = \frac{1}{183.7}; \qquad 183.7 = \frac{736}{X}.$$

This computation indicates a gradient of about 4° in the tissues of the guinea pig, exclusive of fur, and a probable true skin temperature of about

35.2°.[4] If we assume equal weights here for skin and rectal values (see footnote) we derive an average body temperature of 37.2° for the guinea pig at 26.7°. Similar computations for 21.1°, 15.6°, and 10.0° are as follows:

T_A	$T_{Surface}$	T_{Skin}	T_{Rectal}	AVERAGE T_B
26.7°	31.1°	35.2°	39.2°	37.2°
21.1°	26.4°	31.3°	38.7°	35.0°
15.6°	21.7°	27.4°	39.0°	33.2°
10.0°	17.1°	23.6°	38.9°	31.2°

These values illustrate, in a trifle more than principle, the course of events in the adjustments in the zone of chemical regulation. Similar computations could be made for the mouse and rat with the probability that we would find a more rapid decline in the predicted average body temperature, particularly in the mouse. It would be very interesting to know to what extent the mouse, which is accommodated to wide transient fluctuations in body temperature as a consequence of its low thermal inertia, would be able to better the other species in surviving low mean body temperatures. That the possibility of survival at rather low rectal temperatures is wider than is normally thought is certainly true for the rat. Hypophysectomized rats with a metabolism 30 to 40 per cent below normal and rectal temperatures averaging 33.5° to 34.5° have been frequently observed, and quite recently it has been shown that 31°C. to 32°C. levels of rectal temperature may be maintained in human beings for days. By survival temperatures we do not mean temperatures from which the animal may be revived, but temperatures to which it successfully adapts for considerable periods of time.

It seems useful, in this connection, to point out the decided error in concluding that lowered rectal temperatures indicate, per se, a loss of temperature regulation. The operated animals mentioned above have what appears to be a normal type of regulation except that the point of thermal neutrality is usually 2° to 6° above 28° and the degree of chemical regulation, as compared with normal members of the species, is roughly proportional to their lowered metabolism. The fact that the absolute limits of their range of adaptation in terms of the common physical scale of temperature is restricted, is confusing rather than important. The heat regulation of such animals can be properly examined only in terms of a

[4] That this value is approximately correct has been demonstrated through rectal thermocouple readings at 8, 6, 5 and 2 cm. (39.2°, 38.0°, 37.6°, and 35.6°) in a fasting guinea pig at this temperature, and through threading a fine thermocouple into a very shallow lateral channel in the skin made with a small needle. An extrapolation of the rectal readings would indicate a temperature of 34.0° to 34.5° at 0 centimeter.

physical scale of much finer structure. A comment is likewise frequently seen to the effect that the variable rectal temperature of small, warm blooded animals, particularly the mouse, is an indication of a rudimentary type of heat regulation, the animal being in part poikilothermous. It seems to us that such conclusions are scarcely valid. They certainly confuse, in some part, the distinction between the degree to which a type of physiological function is present, and the objective physical consequence of various degrees of thermal inertia and susceptibility in relation to a physical temperature scale.

These small species show conspicuous chemical regulation but the increased heat production never restores the body of the animal to the same thermal state (identical skin temperature, rectal temperature, and average body temperature) which existed under warmer conditions. In this respect the small animals' adjustment to cold has a consequence similar to that seen in humans, namely, a lowered average body temperature, and this in spite of a pronounced increase in heat production in the animal.

It was a matter of surprise to find that the chemical regulation appearing in these small animals as a defence against a given degree of cold is obviously not a very complete homeostatic adjustment (as judged in terms of average body temperature) in spite of the fact that the heat-generating capacity of the animal has unutilized reserves which might be drawn upon. For a drop of 1°, the mouse generates approximately 46 Calories of heat over its metabolism at the warmer temperature and suffers a small fall in average body temperature which becomes significantly large for decreases of 5° to 10°. Potentially, however, the mouse could produce several times this amount of heat, as is clearly seen from the metabolic results at 10° and 15° air temperature. From an energy standpoint, the evaporative mechanism for human heat regulation in the hot zone is of an equal order of potency, and is likewise not completely utilized to prevent rise in average body temperature. Although these apparently non-adaptive changes in average body temperature are in opposite directions, they seem to be comparable insofar as the broad features of heat regulation are concerned.

It is possible that the physical factor of internal conductance is concerned in the failure of the small animal to employ what appears to be its potential metabolic power in the adaptation. Regardless of the species, it is unusual to find, in warm blooded animals, a difference of less than 1.5° to 2° between rectum and skin, and conceivably this represents something in the nature of a minimum gradient per unit of normal metabolism under maximum conditions of peripheral blood flow. It is certainly true that the heat production of animals is concentrated to a degree internally and that the convective efficiency of blood flow never approximates that of an infinitely well stirred water bath. Hence, large increases in heat production with the maintenance of a constant skin temperature in pro-

gressively colder environments might well entail large rises in the deepest body temperature. As an illustration we may take the case of the guinea pig at 29.5° air temperature. At this point the guinea pig is at the threshold of overheating. Further rises of environmental temperature obviously cannot be compensated by circulatory changes since rectal rises occur in spite of rudimentary evaporative regulation (wetting of the fur) and hyperpnea. At this point computation on the basis previously used indicates a rectal-true skin differential of about 2°. If the same skin temperature were maintained at 24° air temperature with the same rectal-true skin conductance as at 29.5°, it would necessitate a rectal temperature of approximately 41.5°. At lower air temperatures this computed rectal value would rise to impossible heights. It might be supposed that the conductance would be increased proportionately with the increase in metabolism and general circulation with a resulting constant true skin-rectal difference. Considerable evidence can be assembled, however, from studies in the physiology of exercise, and temperature observations of fever and artificial hyperpyrexia to indicate that this is not likely.

In any case, it seems clear that a regulative mechanism which, in a cold environment, would actually restore the skin to some ideal thermal state must inevitably produce undesirably high internal body temperatures; while a mechanism which maintains an ideal temperature in a variable internal zone must necessarily leave surface temperature low. Actually, the human being and the small animal alike—after minimum conductance is attained—can effect only a partial adaptation in which both surface and internal temperatures are below the optimum. It seems probable that the mechanisms of heat regulation preserve the status quo only within a relatively small portion of the total temperature range to which survival adaptation is possible. In this factor, as well as in the effects of the specific physiological costs of adaptation, are the probable causes of longer term temperature effects and new levels of adaptation.

CONCLUSIONS

1. An extensive study of the metabolism of guinea pigs, rats, and mice has identified a region of thermal neutrality at 30 to 31° for the guinea pig, 28 to 29° for the rat, and between 30° and 33° for the mouse. Metabolisms of the order of 590, 680, and 740 Calories per square meter per 24 hours, in the same species order, are typical of these temperature ranges.

2. Above this critical area, the metabolism under hot conditions is very variable, due possibly to the lack of a true physiological regulation by evaporation. All three species make an effort at adaptation through spreading saliva on their coats, but the success of this process is highly variable.

3. It has been shown that the metabolic increment with decrease in environmental temperature is species-specific and highly regular. The value of this increment, however, is not in 1:1 correlation with susceptibility to heat loss as judged by surface-weight ratios, and reflects differences in peripheral insulation and probably in response adequacy.

4. Chemical regulation in these animals is not as completely effective as has been thought. It appears that in spite of large increases in metabolism, the average body temperature falls with decreasing environmental temperature in much the same manner as has been observed in larger animals which do not show chemical regulation in this range. This fall in average body temperature produces failure of regulation when the physiological limit of storage is reached.

5. Values are given for surface temperatures in conjunction with known levels of rectal temperature, air temperature, and metabolism. From these values the various conductances, including the environmental heat loss constants, have been computed. Derived values for average body temperature are presented as an illustration of the gradual failure of heat regulation in progressively colder environments.

6. Theoretical considerations are presented in regard to the practical limit of chemical regulation which suggest that such a limit is not entirely dependent upon the reserve of heat producing capacity.

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THE EFFECT OF ASCORBIC ACID ADMINISTRATION UPON EXPERIMENTAL POLYCYTHEMIAS: THE MECHANISM OF COBALT POLYCYTHEMIA

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The production of an experimental polycythemia by the continued administration of cobalt or its salts was first demonstrated by Waltner (1) in rats, Kleinberg (2) in rabbits, and Mascherpa (3) in the dog. Barron and Barron (4) have reported that the daily intravenous injection of ascorbic acid depresses cobalt polycythemia in rabbits, or, if administered simultaneously with cobalt, prevents entirely the appearance of the polycythemia. They showed also that red blood cells from rabbits with cobalt polycythemia had a higher rate of oxygen consumption than cells from normal rabbits or humans with polycythemia vera; and that the addition of a small amount of cobalt to these red cell suspensions "in vitro" caused a marked inhibition of their respiration. They ascribe the production of cobalt polycythemia to an inhibition of the respiratory function of immature red cells in the bone marrow and their subsequent early release into the general circulation as mature cells.

The present investigation was undertaken to see whether we could confirm, in dogs, the results of Barron on the effect of ascorbic acid upon cobalt polycythemia; and to study, in addition, the effect of ascorbic acid upon the polycythemia induced in dogs by daily exposure to a low environmental air pressure.

PROCEDURE. The dogs used in these experiments were fed a constant diet of Purina dog chow.

Polycythemia was produced in two dogs by the feeding of 2 mgm. of cobalt, in the form of cobaltous chloride, per kilogram of body weight per day. Two other dogs received daily exposure to low atmospheric pressure (430 mm. of mercury) for six hours. Two dogs received, first, the daily low pressure chamber stimulation, and when a steady state of polycythemia had been induced thereby, received, in addition and simultaneously the daily cobalt administration.

The low pressure chamber[1] used in these experiments is generally cylin-

[1] We wish to express our gratitude to the Department of Physiology of the University of Chicago for the loan of the pressure chamber and vacuum pump used in these experiments.

drical in shape, being about three feet in diameter and about three feet high. The outlet pipe of the chamber is connected to a motor driven vacuum pump, while the inlet pipe, with an adjustable valve attached, communicated with the air of the room. Hence the air within the chamber which provided the dog's atmosphere was being continually replaced by fresh room air. By adjusting the air intake valve, a constant pressure was maintained at 430 mm. of mercury.

Blood samples were drawn from the saphenous vein while the dog was in an unexcited state, and at least 15 hours after the previous daily administration of cobalt or low pressure exposure. Erythrocyte counts, leukocyte counts, hemoglobin determinations (Sahli), and reticulocyte percentages by Wakerlin's method (5) were made at frequent intervals.

A number of determinations of the reduced ascorbic acid content of whole blood were made. Blood filtrates were prepared as described by Stephens and Hawley (6) and titrated against 2,6 dichlorphenol-indo phenol according to the method of Birch, Harris, and Ray (7). All titrations for vitamin C were performed in duplicate or triplicate. Fresh dye solutions were prepared from standard tablets made by Hoffmann-La Roche, Inc.

After polycythemia had been established, ascorbic acid[2] was fed to the dogs in a daily dose of 8 mgm. per kilogram of body weight.

RESULTS. Figure 1 shows the development of experimental polycythemia in two dogs (1 and 2) by cobalt administration, and in two other dogs (3 and 4) by daily exposure to low atmospheric pressure—and the effect thereon of subsequent administration of vitamin C. The daily oral administration of 8 mgm. of ascorbic acid per kilogram of body weight, in addition to cobalt, to dogs 1 and 2 caused prompt reduction of their erythrocyte numbers almost to their pre-cobalt levels. In both dogs, blood hemoglobin and reticulocyte percentages were reduced also, while the leukocyte counts did not change significantly. When the administration of ascorbic acid was discontinued, 8 and 14 days later respectively, the erythrocyte numbers and hemoglobin percentages returned to their polycythemic values.

Feeding of the same daily dose of ascorbic acid to dogs 3 and 4 (fig. 1) which were made polycythemic by daily exposure to low air pressure, *did not* cause any significant change in the red blood cell counts or hemoglobin.

The additive effects of low air pressure and cobalt on the erythrocyte numbers of two dogs are shown in figure 2. When 8 mgm. of ascorbic acid per kilogram of body weight were fed daily to these dogs, their polycythemias were *partially reduced* within a few days. We then doubled the daily vitamin dose for one of the dogs in an effort to reduce further his

[2] The Abbott Laboratories very kindly furnished us with crystalline, synthetic vitamin C, in tablet form.

red cell count, but in this we were not successful. Figure 2 shows distinctly that ascorbic acid abolishes the increase of red cells that is induced

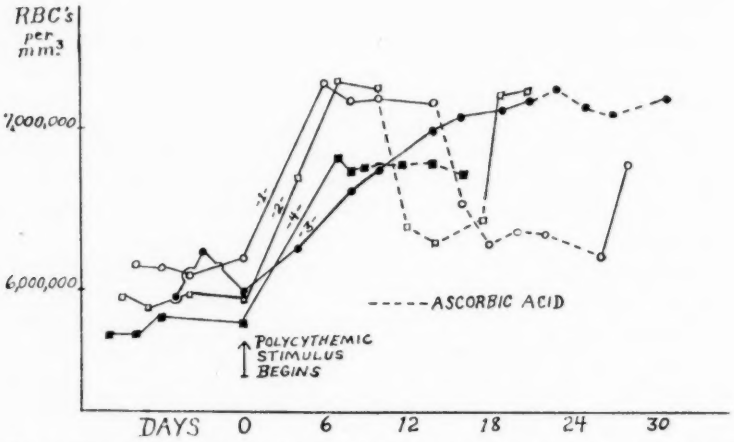

Fig. 1. The influence of ascorbic acid feeding upon experimental polycythemias. Cobaltous chloride is the polycythemic stimulus to dogs 1 and 2, while dogs 3 and 4 were exposed for 6 hours daily to low atmospheric pressure (430 mm.).

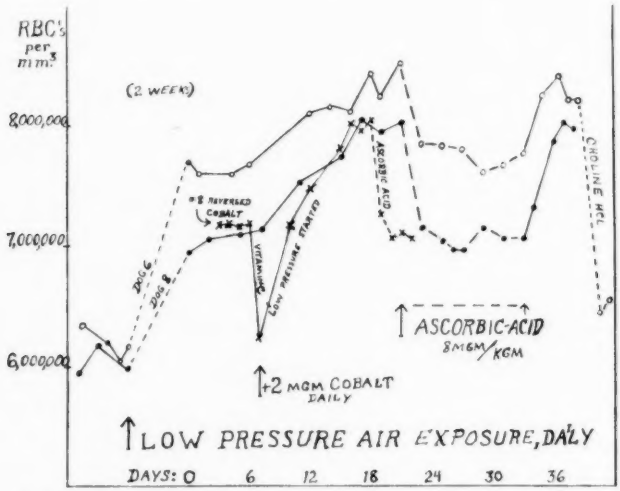

Fig. 2. Showing the additive effect of cobalt and low atmospheric pressure in producing polycythemia; and its partial depression by orally administered ascorbic acid.

by cobalt, but has no effect upon that portion of the polycythemia which is produced by exposure of the animal to low atmospheric pressure. The shortest line in figure 2 shows an experiment on dog 8 in which the procedure for producing a summation of polycythemia was reversed, i.e., cobalt feeding was commenced prior to low pressure exposure.

A number of vitamin C determinations were made on blood from dogs with cobalt polycythemia. Blood which was drawn one hour after the daily dose of cobalt had been fed was found to contain quite uniformly, about 0.08 mgm. per cent less ascorbic acid than before cobalt feeding.

Table 1 shows the effect of adding cobalt in a concentration of 1 mgm. per cent to blood upon its ascorbic acid content. In these experiments, 0.05 cc. of a 1:5000 solution of cobalt (in the form of $CoCl_2 \cdot 6H_2O$) was added (per cc.) to one portion of a blood sample, while an equal volume of untreated blood from the same sample was used as a control. The bloods

TABLE 1

Effect of adding 1 mgm. per cent of cobalt, manganese or nickel to dog's blood, in vitro, upon its ascorbic acid content

Reduced ascorbic acid is tabulated in milligrams per 100 cc. of blood

KIND OF DOG'S BLOOD	UNTREATED BLOOD	COBALT ADDED	MANGANESE ADDED	NICKEL ADDED
Normal	0.74	0.66		
Normal	0.62	0.54		
Cobalt polycythemia	0.70	0.56		
Cobalt polycythemia	0.70	0.57		
Cobalt polycythemia	0.68	0.55	0.65	0.68
Cobalt polycythemia	0.66	0.54	0.67	0.67

were agitated occasionally over a five minute period, then treated with trichloracetic acid, filtered, and titrated. In a few experiments, equivalent amounts of manganese or nickel were added to polycythemic blood in place of cobalt.

In these experiments, the addition of cobalt caused a significant (20 per cent) reduction in the ascorbic acid content of polycythemic blood, but equivalent quantities of manganese or nickel had no such effect (table 1). The latter elements were added to the blood in the form of manganous sulphate and nickel nitrate.

It may be proper to mention here that we have fed a daily dose of 2 mgm. of cobalt per kilogram to 3 dogs over a period of five months, that polycythemia was produced and maintained during this period, and that no untoward effects upon the animals were observed.

DISCUSSION. The reduction of cobalt polycythemia in our dogs by ascorbic acid administration (fig. 1) generally confirms similar findings by Barron and Barron (4) on rabbits. Ascorbic acid was administered *orally*

in our experiments, and a considerably smaller "per kilogram" daily dose was found to be effective.

The fact that vitamin C does not reduce the high red cell counts induced by low atmospheric pressure (fig. 2, and dogs 3 and 4, fig. 1) certainly indicates that the mechanism of production of this type of polycythemia is different from that of cobalt polycythemia. A difference in mechanisms is also indicated by the summation of polycythemia by the two different stimuli (fig. 2). Previous experiments (8) show that cobalt polycythemia cannot be intensified even when the daily dose of cobalt is increased to three times that used in these experiments.

We have shown previously (9) that the daily oral administration of 8 mgm. of choline hydrochloride per kilogram of body weight, or of 75 grams of beef liver per 10 kgm. dog, will depress either of the two experimental types of polycythemia used in this work. But, in the two dogs with combined (cobalt plus low atmospheric pressure) polycythemia which we have thus far studied, choline administration has not depressed the red cell counts completely to their normal values. Since our low pressure polycythemias have been generally greater in size than those induced by cobalt, it appears that choline is particularly effective in depressing this type. In emphasizing a difference in mechanisms of polycythemias, it is of interest to note that human polycythemia vera, according to Kandel and LeRoy (10), is not ameliorated by the daily administration of 400 mgm. of ascorbic acid.

The fact that cobalt, fed to polycythemic dogs or added to their blood in vitro, *lowers* the concentration of reduced ascorbic acid—suggests that cobalt may tie up the vitamin or interfere with its functioning. The antagonism of the erythropoietic action of cobalt by the feeding of vitamin C also may be viewed as supporting this idea.

Since Barron and Barron (4) have shown that the addition of cobalt, in vitro, to red cell suspensions from polycythemic rabbits diminishes the oxygen consumption of these cells, the question arises: "Does cobalt directly impair the respiration of these cells or (2) does it interfere with a possible respiratory function of ascorbic acid and thereby *indirectly* impair the oxygen consumption of immature red cells?" We believe that the second suggestion is correct, that ascorbic acid plays a vital part in the respiration of these cells. If the vitamin merely served to "detoxify" or to bind cobalt, we would expect it to bind similarly such elements as nickel and manganese which are rather closely related to cobalt. But nickel nitrate and manganous sulphate when added to polycythemic blood, in vitro, did not change its ascorbic acid content. Neither of these elements is known to produce polycythemia.

Furthermore, the rapidity with which ascorbic acid feeding reduces

cobalt polycythemia (48 hrs.) makes it appear unlikely that the vitamin acts merely by binding cobalt, especially when we consider that cobalt polycythemia does not usually subside until at least one week after cessation of cobalt administration (8).

CONCLUSIONS

Cobalt administration and anoxia show additive effects in the production of a marked experimental polycythemia.

Ascorbic acid, in a daily oral dose of 8 mgm. per kilogram of body weight, depresses cobalt polycythemia in dogs but does not affect the polycythemia produced by daily exposure to low atmospheric pressure.

Cobalt diminishes the ascorbic acid content of blood from dogs with cobalt polycythemia.

The theory of Barron and Barron on the mechanism of cobalt polycythemia is generally supported by our experiments. We suggest, however, that cobalt may stimulate erythropoiesis by interfering with a respiratory function of vitamin C. Some evidence for this is presented.

Cobalt, in a daily dose of 2 mgm. per kilogram has been administered orally to 3 dogs over a period of 5 months. Polycythemia was produced and maintained, and no untoward symptoms were observed.

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THE SECRETION OF AMMONIA BY THE SMALL INTESTINE OF THE DOG[1]

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In 1935 we reported (1) the finding of large amounts of ammonia in the succus entericus of jejunal fistulae in dogs. A marked reduction in the concentration of fixed base in the juice accompanied the increased ammonia content. In a preliminary report (2) it was stated that although acidosis did not increase the ammonia of the juice, a meat diet or the administration of urea markedly increased the ammonia content. These studies have been extended and confirmed (3). Recently Ingraham and Visscher (4) reported that ammonia production occurred in the intestine during active absorption involving osmotic work. Poisons which abolished active absorption, greatly diminished ammonia accumulation. This report is concerned with a study of the various factors which influence the concentration of ammonia in the juice secreted by a Thiry-Vella loop of jejunum in the dog.

METHODS. Healthy dogs with Thiry-Vella fistulae made in the first portion of the jejunum were used. The technique of collection and chemical analysis of the juice was similar to that previously described (3). To minimize the error due to products of bacterial activity, the loop was washed with warm saline and the first portion of succus entericus discarded. Graph 1 portrays one of six experiments performed on six dogs. These involved a quantitative study of the amount of protein ingested, the daily urinary excretion of ammonia, urea and creatinine and the ammonia content of the intestinal juice. The dogs were kept in metabolism cages except when juice was collected. The cages were thoroughly washed and any urine samples, showing fecal contamination, were discarded. The dog biscuits were fed ad libitum. The carbohydrate diet consisted of starch and was fed at a level to furnish twice the estimated basal caloric requirement. After the period of carbohydrate feeding, 1.5 grams of protein per kilo of body weight in the form of lean beef was substituted for some of the carbohydrate, the estimated caloric intake being kept constant. After this, the protein content was increased to 5 grams per kilo of body weight, the estimated caloric intake remaining constant.

[1] Supported in part by a financial grant from the Wisconsin Alumni Research Foundation.

After this period the protein intake remained constant and the carbohydrate was increased to furnish twice the estimated caloric requirement. For the final period lean meat entirely replaced the carbohydrate, with the estimated energy intake twice the basal requirement.

RESULTS. The particular method used to secure samples of intestinal juice made it imperative to run many control experiments. The ammonia

TABLE 1

Influence of various diets upon the ammonia content of daily collections of intestinal juice

DIET	AMMONIA CONTENT OF INTESTINAL JUICE, MILLIGRAMS PER 100 CC.					
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Purina dog chow	38.6	20.1	21.2	20.4	17.0	20.4
	20.4	8.0	10.6	42.7	34.0	28.4
	27.6	8.7	18.7	23.8	27.2	22.1
	27.6	14.9	21.2	29.7	28.9	
	29.3	11.9			23.8	
Average	28.7	12.7	22.9	29.1	26.2	23.6
Carbohydrate	11.0	17.0	5.1	12.9	12.7	11.9
	9.3	12.7	5.9	7.6	13.6	10.2
	8.5	5.1	5.1		10.2	8.9
	2.0	10.2	7.6		7.6	8.9
	5.1	5.1	5.1		2.2	10.2
Average	7.2	10.0	5.7	10.2	9.3	10.0
Meat	40.8	34.0	22.1	66.3		
	23.8	31.4	21.6	54.6		
	52.8	33.1	34.8	56.0		
	36.7	42.5		71.4		
	43.3	44.2	29.7			
	37.4		31.8			
	52.2					
Average	41.0	38.8	28.0	62.1		

in the juice might arise from bacterial action in the intestinal contents. This was minimized by the preliminary washing. Solutions of urea or amino acids were slowly infused into the cephalic end of the fistula and the contents escaping from the other end were found to have a much lower ammonia content than the succus entericus collected immediately prior to the perfusion. This demonstrates that at least in the period of juice collection there is no factor in the loop's contents which would form ammonia from the most likely sources, namely, urea and amino acids. Juice could

be supplemented with urea and kept at 25° for 24 hours without any increase in ammonia. It could be kept in the cold room with the addition of toluene for 2 days without any change in the content of ammonia. These two observations render it extremely unlikely that there is present in the succus entericus any enzyme or other factor which liberates ammonia, within a reasonable period of time. Furthermore, the composition of the succus entericus might be modified by the intestinal absorption of some of its constituents. This factor was not controlled in these experiments.

The protein content of the diet greatly influences the ammonia content of the juice (table 1). Changing from Purina dog chow which was about

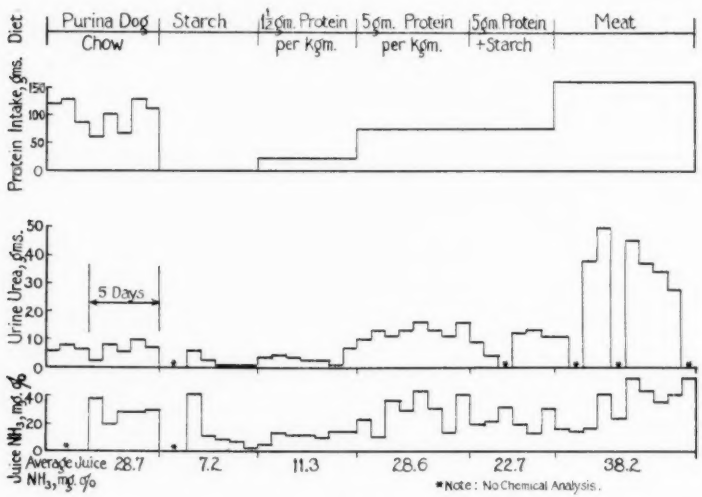

Fig. 1

23 per cent protein, to a carbohydrate diet resulted in an average reduction of ammonia content of 21 to 75 per cent. When the diet was changed from carbohydrate to meat, the increase in ammonia content averaged 3.8 to 6 fold. In figure 1, it is seen that the ammonia content of the juice follows changes in the urinary nitrogen excretion rather than the amount of nitrogen ingested. The addition of carbohydrate to the meat diet reduced both urinary nitrogen and the ammonia content of the juice. The carbohydrate, by its protein sparing effect, has lowered protein metabolism. A similar result was obtained in the comparison of the results obtained with feeding two kinds of dog biscuits. Purina dog chow and Champion dog biscuits have about 23 per cent protein but the fuel

value of the former is 1400 calories per pound and for the latter 2300 calories. The juice ammonia with the former diet in 37 observations averaged 27.5 mgm. per cent but with the Champion biscuit it was always less than 18 mgm. per cent. These results indicate that the ammonia content of the juice is determined more by protein metabolism than by the amount of protein ingested.

In table 2, it is seen that the intravenous injection of glycine or alanine or glutamic acid markedly increased the ammonia content of the juice. These 3 amino acids gave about the same result.

It is seen in table 3 that in 18 of 21 experiments, the administration of urea resulted in a marked increase in the ammonia content of the juice. In most cases the increase in the ammonia content of the juice does not parallel the increase in blood urea. Therefore, one cannot say that there

TABLE 2

Influence of the intravenous injection of amino acids upon juice ammonia

Milligrams of NH_3 per 100 cc. of juice

DOG Y2		DOG 7		DOG Y3		DOG Y4		DOG 8	
Before	After	Before	After	Before	After	Before	After	Before	After
7.6	15.3	17.0	23.8	10.2	23.8	15.3	31.4	18.7	22.1
21.2	32.3	30.6	32.3	11.0	18.7				
12.7	20.4								

DOG Y1		DOG 9		DOG 10		DOG Y5	
Before	After	Before	After	Before	After	Before	After
8.5	14.4	17.0	42.3	4.0	9.5	Trace	22.0
5.1	24.6	19.5	28.0	1.4	3.7	8.1	15.0

is a direct relationship between these two factors. In intestinal obstruction, peritonitis and Addison's disease, characteristically the blood-chlorides are reduced and the blood urea elevated. Table 3 shows the results of the intravenous injection of 10 grams of NaCl after the blood urea had been elevated. The data particularly of dogs 9 and 11 suggest that the NaCl tended to prevent the usual increase in the ammonia content of the juice.

Inasmuch as a protein diet would result in considerable urea production the question arose as to whether the effect of such a diet upon the juice ammonia might be exerted through the urea production. However, it was found that extensive diuresis after a protein meal largely prevented the elevation of blood urea but the ammonia content of the juice showed the usual increase. Also, the elevation in the blood urea during the ex-

periments with the amino acid injection was not significant. Therefore, the meat diet and the amino acids exert their effect upon the ammonia content of the intestinal juice in some other way than by the blood urea.

The experimental studies thus far had led to the idea that the ammonia in the intestinal juice was a product of protein metabolism in the intes-

TABLE 3

Relation between the concentrations of blood urea and juice ammonia. Influence of NaCl upon this relationship

Concentrations are expressed in milligrams per 100 cc.

PROCEDURE	DOG 2		DOG 8		DOG Y1		DOG Y3		DOG Y2	
	B.U.	Juice NH_3	B.U.	Juice NH_3	B.U.	Juice NH_3	B.U.	Juice NH_3	B.U.	Juice NH_3
Normal	20	4.2	32	2.5	27	5.8	28	11.0	29	4.2
Urea admin.	105	4.2	182	2.5	116	12.7	145	22.1	121	15.3
Normal	25	5.9	6	11.7	20	7.6	32	23.8	24	5.9
Urea admin.	131	6.9	116	18.7	135	9.3	147	41.6	128	13.6
Normal					32	2.5	30	7.6	29	3.4
Urea admin.					155	17.8	181	13.6	140	26.3

	DOG 6		DOG 7		DOG 11		DOG 9	
	B.U.	Juice NH_3	B.U.	Juice NH_3	B.U.	Juice NH_3	B.U.	Juice NH_3
Normal	45	6.8	25	23.8	18	16.1	29	10.2
Urea admin.	118	104.5	109	52.7	145	28.9	156	30.6
Normal	33	7.6	20	12.7	22	14.4	20	2.5
Urea admin.	156	80.7	111	51.8	87	24.6	165	7.2
Normal	27	19.5	14	7.6				
Urea + NaCl	112	31.4	98	13.6				
Normal	27	15.3	17	3.4	30	12.0	17	8.5
Urea + NaCl	113	28.0	109	13.1	107	10.2	68	9.0
Normal	32	34.9	12	13.6	19	5.9	15	23.8
Urea + NaCl	98	42.5	98	28.0	104	6.8	72	17.8

tinal glands. Insulin and adrenalin are two hormones, generally believed to exert a direct effect upon the metabolism of carbohydrate and therefore indirectly upon protein metabolism. Representative data of 15 experiments upon the effect of insulin are seen in table 4. The dose of 10 units of insulin in dog 9 produced very clear cut results. Initially there was a 10 per cent decrease in the ammonia content followed by a very great in-

crease, at which time the dog was in hypoglycemic shock. This degree of hypoglycemia was not produced in the other dogs. In only 4 of the 15 experiments was there a decrease in juice ammonia. It was expected that this would be, at least, the initial effect of insulin because the tissues would have an increased carbohydrate metabolism. The elevation of the juice ammonia which occurred in 12 experiments might be due to the increased glyconeogenesis from protein as a result of the hypoglycemia. In this connection it is interesting to note the report of Kiech and Luck (5) that insulin increased the rate of urea formation in rats.

The intravenous injection of adrenalin always produced a significant change in the ammonia content of the juice but the results were extremely variable. Seven dogs were used. In 5 experiments the ammonia con-

TABLE 4

The effect of insulin upon the ammonia content of the intestinal juice

DOG	INSULIN DOSAGE	AMMONIA CONTENT OF JUICE (MGM. PER 100 CC.)		
		Normal	After insulin	After insulin
	units			
9	10	16.1	14.5	43.0
	5	9.4	11.9	13.6
2	5	9.4	14.5	24.6
	5	6.8	12.8	13.6
1	7.5	17.0	15.5	
	7.5	19.0	19.0	
	5.0	11.1	12.9	15.3
4	7.5	21.3	34.9	
	7.5	18.5	10.0	
	15.0	10.2	14.5	7.7
5	8	11.0	18.7	
11	8	7.7	16.2	

tent first decreased and later increased above the normal. In 14 experiments the ammonia content increased and in 12 experiments it decreased. The dosage of adrenalin varied from 1 cc. to 3 cc. of 1:1000 adrenalin and was given intravenously. Generally when the 3 cc. dose was administered in small portions over a period of 60 to 90 minutes, the ammonia content of the juice decreased. However, in 6 experiments, adrenalin 1:250,000 was intravenously injected at a constant rate of 1 cc. per minute and the ammonia content of the juice increased in 4 cases. Youmans (6) found that this dosage was slightly above the threshold for inhibition of intestinal movements. The cause for this variable experimental result is not apparent. Although adrenalin is generally believed to increase carbohydrate metabolism (7) Luck and Morse (8) have reported that adrenalin lowers the blood amino acid nitrogen. This suggests that

adrenalin has stimulated protein metabolism. If adrenalin exerts these two effects, the variability in the experimental results is comprehensible.

Discussion. Ammonia has been found in the intestinal contents by a number of workers. Bliss (9) found much ammonia in the vomitus and duodenal contents of nephrectomized dogs. The venous blood from the first portion of the small intestine in these dogs was very rich in ammonia. In such dogs, blood urea would be very high and one would therefore expect much ammonia in the intestinal juice. Herrin (1) found considerable ammonia in the juice escaping from distended jejunal fistulae. Hibbard (10) found much ammonia in the intestinal contents of bowel obstruction in dogs. The origin of the ammonia in these observations might well be putrefaction within intestinal contents. However, in the more recent reports of this laboratory (3) and of Visscher (4) isolated loops of intestine were used and after a preliminary washing with saline the observations only extended over a period of 2 hours. These procedures would render it unlikely that the ammonia arose from putrefaction. Furthermore, Roese (11) found that the blood perfusing a loop of dog's intestine contained considerable ammonia. The blood ammonia was markedly increased by introducing glycine into the lumen of the bowel, and also by the addition of glycine or urea to the perfusing blood. London and associates (12) also reported that the injection of amino acids increased the ammonia content of the blood leaving the intestine. These reports indicate that ammonia may be formed by the tissues making up the intestinal wall. The control experiments in this study gave no evidence of any factor which might liberate ammonia from urea or amino acids within the lumen of the fistulous loop. The effect of the following factors upon the ammonia content of the juice,—diet, intravenous injection of amino acids or insulin or adrenalin and the administration of urea, render it highly improbable that the ammonia in these experiments could have originated from changes within the intestinal contents. The blood could hardly be considered as a source of the ammonia because of its very low ammonia content. The most likely origin of the ammonia is the glands of the jejunal mucosa.

The physiological significance of the ammonia in the succus entericus is of some concern. The results obtained with the diets and the amino acid experiments might well be interpreted on the basis that the ammonia arises from the protein metabolism of the glands. The results with insulin and adrenalin could be explained on this basis although there are other possibilities. There is no apparent reason for believing that urea would increase protein metabolism. Consequently no explanation is forthcoming for the experimental results with urea or the combination of urea and NaCl. This idea of a metabolic origin of the ammonia would demand the presence of deaminase and urease systems in the glandular cells.

Bollman and Mann (13) have reported that the ammonia content of the blood increases after hepatectomy and complete nephrectomy in the dog. The elevation was less when these operations were combined with almost complete removal of the gastro-intestinal tract. They suggested that fermentation, in the intestinal contents, had furnished ammonia to the blood stream. On the other hand, the observations of Roese (11) and London (12) suggest that the tissues of the gastrointestinal tract might have contributed considerable ammonia to the blood. There is no reason for believing that the ammonia itself is elaborated for a purpose such as seen in renal function. In an earlier report (3), it was clearly demonstrated that acidosis would result in a lowering of the fixed base in the intestinal juice without any increase in ammonia. Because of all these observations and considerations it would seem that the ammonia in the succus entericus has its origin in the metabolic activities of the gland cells, particularly those involving protein metabolism. The ammonia itself is produced for no particular purpose inasmuch as it is not involved in a conservation of sodium. It is a product of metabolism and only incidentally appears in the succus entericus because of its great diffusibility. The changes in the ammonia content of the intestinal juice are believed, therefore, to be a reflection of metabolic processes within the glandular cells.

SUMMARY

Ammonia is found in the succus entericus from a Thiry-Vella loop of jejunum in the dog. The concentration of ammonia in the juice is influenced by a number of factors. When the diet was changed from dog biscuit which contained 23 per cent protein to carbohydrate, the average ammonia content of the juice decreased 27 to 74 per cent in 6 dogs. In 4 dogs the change from a carbohydrate to a meat diet resulted in a 3.8 to 6 fold increase in the ammonia content. In metabolism experiments with 6 dogs, marked changes in the ammonia content of the juice paralleled changes in urinary nitrogen excretion rather than nitrogen intake. The urinary nitrogen excretion was changed either by varying the amount of protein ingested and maintaining a constant caloric intake or by increasing the carbohydrate intake and maintaining the amount of ingested protein constant.

The intravenous injection of glycine or alanine or glutamic acid in 14 of 17 experiments on 9 dogs resulted in a 42 to 164 per cent increase in the ammonia content of the juice. In two experiments, the increase was much greater and in one there was no significant change.

The administration of urea was the most effective means of elevating the ammonia content of the juice. In 10 of 21 experiments on 9 dogs the per cent increase in the juice ammonia ranged from 22 to 130 and in

8 experiments the increase was much greater. In 10 experiments the intravenous injection of 10 grams of NaCl apparently partially checked the effect of an elevated blood urea upon the juice ammonia. This is seen particularly in dogs 9 and 11. The protein diets probably did not increase the juice ammonia by elevating blood urea because extensive diuresis largely prevented the elevation of blood urea without interfering with the increased ammonia content of the juice. In the amino acid experiments the elevation of blood urea was not significant.

The injection of insulin in 12 of 15 experiments resulted in a marked increase in the juice ammonia. Adrenalin gave highly variable results. In 12 experiments the ammonia content of the juice decreased and in 14 it increased.

The interpretation of the above experimental observations is that the ammonia is secreted by the glands of the jejunum. Its concentration in the juice is determined largely by the protein metabolism of the gland cells. The ammonia itself apparently serves no physiological purpose and only appears in the juice and the blood from the intestine because of its formation and its diffusibility.

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EFFECT OF ADRENALIN ON PULMONARY VENTILATION: PROPORTIONALITY WITH DOSE

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This report describes the effect on the pulmonary ventilation of chloralose-anesthetized cats of 5-minute, intravenous injection of adrenalin administered at various rates from minimally effective to maximally tolerated. It is thus companion to three predecessors which have dealt with the hyperglycemia (Griffith, Lockwood and Emery, 1939a), blood lactic acid (Griffith, Lockwood and Emery, 1939b) and oxygen consumption (Griffith, Emery and Lockwood, 1930) of these same animals as a result of the same injections.

PROCEDURE. Details of anesthesia and other preparation of the animals may be found in the references just cited.

Pulmonary ventilation was measured by collection of expired air, through valves attached to the tracheal cannula, in a small, carefully calibrated spirometer. Usually two "normal" determinations, 15 to 20 minutes apart and each approximately 5 minutes in duration (but always the time required to fill the spirometer between marks) preceded injection. Injection, always for exactly 5 minutes, was accompanied by another collection of expired air; the two, as closely as possible, beginning and ending together. Further collections were made to cover the periods approximately 5 to 10, and 25 to 30 minutes after injection was finished. Volume measurements were all corrected to standard conditions (0°C. and 760 mm.).

Injection was by hand from a 5 cc. syringe into a cannulated superficial branch of the femoral vein and always for exactly 5 minutes at the rate of 1 cc. per minute.

For injection, Parke-Davis adrenalin chloride was diluted with neutral, isotonic NaCl solution and administered at the following rates in milligrams per kilo per minute, with the number of experiments in parenthesis: 0.00025 (10); 0.00050 (10); 0.00100 (9); 0.00200 (14); 0.00400 (13); 0.00700 (10); 0.01000 (9).

Control injections of NaCl solution alone (10 experiments) were similar in amount, rate and duration.

RESULTS. *Resting, normal pulmonary ventilation* was extremely vari-

able, ranging from 54 to 192, with an average of 106 cc. per kilo per minute; standard deviation, 30.6; coefficient of variation (standard deviation/mean × 100), 28.6. In this respect, however, it merely resembles other functions of these same animals (loc. cit.) and is probably metabolically determined since it shows very high correlation with the coincident level of oxygen consumption and carbon dioxide output ($r = +0.729$ and $+0.781$, respectively) and is evidently much less affected by body temperature ($r = +0.382$), blood lactic acid concentration ($r = +0.232$) or, reciprocally, by blood pressure ($r = -0.133$).

With such wide variability of resting values it might be suspected that the effect of any specific rate of administration of adrenalin would be conditioned to large extent by the existing normal state of ventilation at the time of injection. There are not enough determinations at any one rate of injection to test this statistically, but inspection does not bear it out; nor do the average normal values of the different injection groups vary enough to be seriously disturbing; they are (with rate of injection in parenthesis) cc. per kilo per minute: 120 (0.00025); 110 (0.00050); 81 (0.001); 83 (0.002); 94 (0.004); 140 (0.007); 110 (0.01). Thus, with the exception of the groups receiving 0.00025 and 0.00700 mgm. per kilo per minute, the normal averages are not too dissimilar; and of these the first is of no significance since the rate of injection is entirely ineffective; and the second fits into the results with so little distortion it cannot be suspected of serious error from this cause.

Comparison of the duplicate normals, 15 to 20 minutes apart, which were obtained with 57 of the 84 animals, shows a very stable metabolic condition preceding injection; the average of the first being 103 and of the second, 102 cc. per kilo per minute.

The effect of control injection of isotonic NaCl. During 5-minute injection of isotonic NaCl at the rate of 1 cc. per minute, and 5 to 10, and 25 to 30 minutes after, pulmonary ventilation was 99, 96 and 106 per cent of normal, respectively; indicating no seriously complicating disturbance is introduced by the experimental procedure itself.

These control values have been used as the initial points for the curves of figure 1.

The effect of adrenalin injection. The data are presented in figure 1 in two ways: part I includes three curves which define the change in pulmonary ventilation during the 5-minute injection period (continuous line), and 5 to 10 minutes (dashed line) and 25 to 30 minutes (dotted line) after injection was completed; the single curve of part II of the figure depicts the approximate total change effected by each rate of injection for the entire 35-minute experimental period, as obtained by summing the products of ventilation minute-volume during injection, and 5 to 10, and 25 to 30 minutes later, when multiplied by 5, 10 and 20 (minutes),

respectively. This is not an exact summation of the area beneath the time curves for each of the injection rates, but gives a value easily calculated and sufficiently accurate for the comparisons intended here.

Threshold. Inspection of figure 1 and particularly the single curve of part II makes it evident that 0.001 mgm. per kilo per minute is the lowest

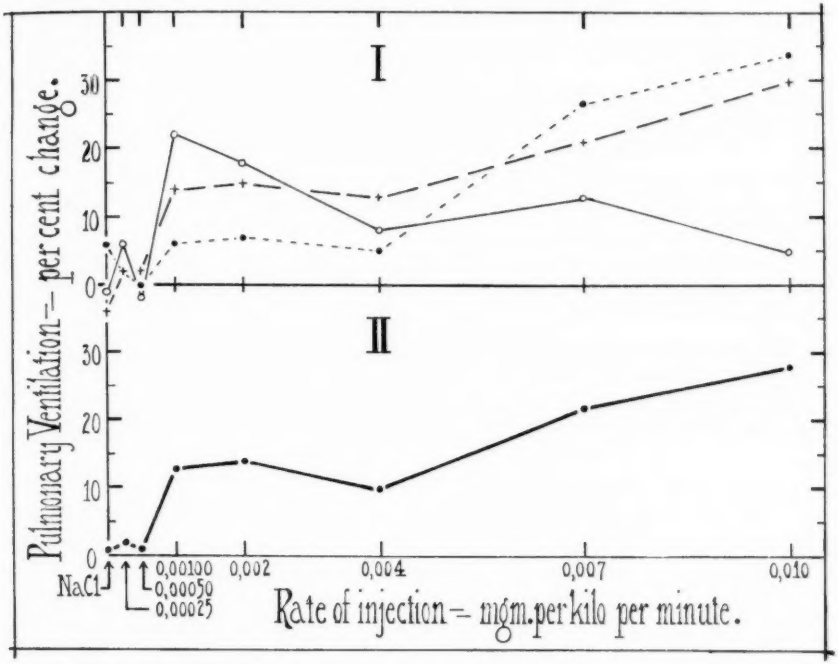

Fig. 1. The effect upon pulmonary minute-volume of intravenous injection of isotonic NaCl solution and of adrenalin at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute for 5 minutes.

I. The upper three curves: Continuous line; during the 5-minute injection period. Dashed line; during the interval 5 to 10 minutes after injection. Dotted line; during the interval 25 to 30 minutes after injection.

II. Total summated change during the 35-minute period including the 5-minute injection and the 30 minutes following.

rate of injection definitely affecting pulmonary ventilation. Blood sugar was definitely, and oxygen consumption slightly but probably increased (+3 per cent) by a rate of injection only half this (previous reports); this slight increase in metabolic rate, however, being apparently without significant measurable effect on pulmonary ventilation. Injection of 0.001 mgm. per kilo per minute was, however, the lowest rate producing appre

ciable increase (+7 per cent) in oxygen consumption and was also quite definitely the threshold for blood lactic acid elevation. These are, of course, necessarily related to pulmonary ventilation and, together with blood pressure, will provide the chief explanation of its variation in what follows.

Proportionality between rate of injection and pulmonary ventilation. Consideration of this relationship will be facilitated by dealing separately with the results obtained: 1, during injection; 2, 5 to 10 minutes; and 3, 25 to 30 minutes after injection; and 4 the total summated effect for the entire 35-minute experimental period.

1. *Pulmonary minute-volume during injection* (fig. 1, I, continuous line): the most outstanding feature of this curve is its complete dissimilarity to any of the effects observed in this series for hyperglycemia, blood lactic acid level or oxygen consumption. It will be seen that maximal increase for this time interval occurs during injection at the threshold rate of 0.001 mgm. per kilo per minute; and, despite minor irregularities, the increase is progressively less at each higher rate of injection.

Without pretending to final understanding of this behavior of the pulmonary ventilation during the 5-minute injection period, it may be discussed in terms of the three variables of undoubted relationship to it: metabolic rate; blood lactic acid level; and blood pressure. Increase of either or both of the first two should increase pulmonary ventilation; increase of the last should depress it.

The two immediately preceding reports of this series have shown that for the 5-minute injection period oxygen consumption is already almost maximally increased during injection at the rate of 0.001 mgm. per kilo per minute and is very similarly increased during the injection itself at all higher rates of administration; lactic acid begins to augment at this same threshold rate of injection and increases progressively as rate of administration is increased. Acting together these two would, therefore, be expected to result in an increase in pulmonary ventilation; which, first apparent with injection at the rate of 0.001 mgm. per kilo per minute, would steadily increase in proportion to dose.

The reason pulmonary ventilation does not follow this expected course must lie in the simultaneous changes of blood pressure. Although record of this was taken at occasional intervals during the injections it was done merely for information as to the effectiveness of injection and the data are not sufficiently complete to describe quantitatively the pressure changes during the 5-minute injection period. Nevertheless they are sufficient to confirm expectation that adrenalin hypertension is, at least approximately, proportional to rate of injection. This, then, would provide a check on pulmonary ventilation, increasing with dose and, as the result shows, apparently able to counteract, to the point of almost complete

extinction, the stimulus to increased ventilation provided by the uniformly increased metabolic rate and the progressively increasing blood lactic acid level.

Further presumptive evidence that pulmonary ventilation during this interval is under complex control would seem to be evident from the

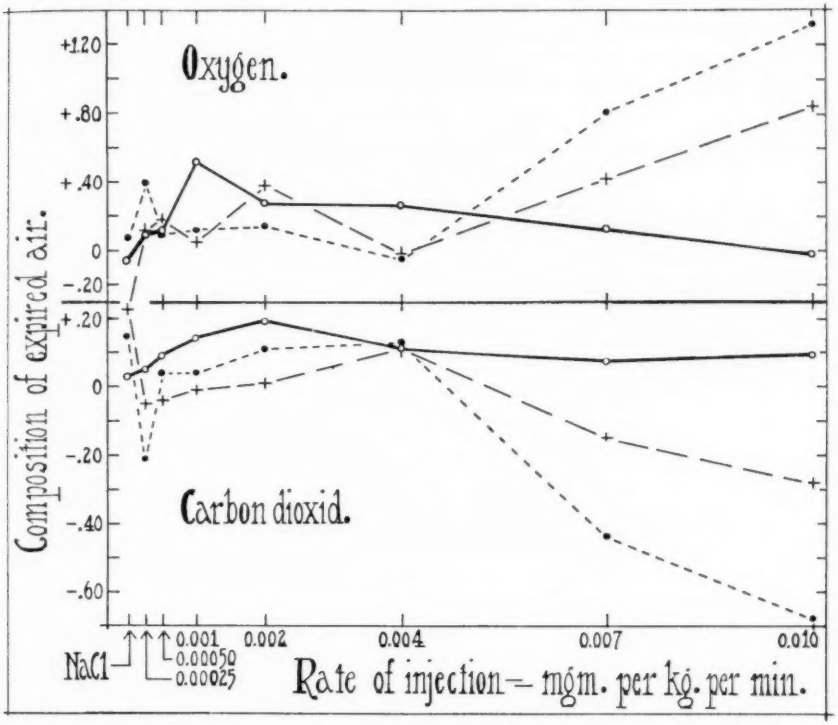

Fig. 2. The change in composition of the expired air resulting from 5-minute injection of NaCl solution and of adrenalin as described for figure 1.

The three curves for oxygen and for carbon dioxide correspond to the three curves of part I of figure 1 and depict the changes occurring during the 5-minute injection (continuous line), and during the intervals 5 to 10 (dashed line) and 25 to 30 (dotted line) minutes after injection.

changes in composition of the expired air which are shown in figures 2 and 3. Unopposed control in behalf of metabolic need, as in exercise, could be expected to result in increased percentage of carbon dioxide (Bock, et al., 1928, and others) and decreased percentage of oxygen in the air expired, or as the latter is usually expressed, increased percentage of oxygen utilization or oxygen deficit of the expired air (Bock, et al., ibid.;

Carpenter and Fox, 1931). The increased percentage of carbon dioxide is seen to be realized (fig. 2, continuous line). This is accompanied, however, not by decrease but by increase in oxygen percentage (fig. 2) and a corresponding decrease in oxygen deficit (fig. 3, continuous line). Apparently, then, pulmonary ventilation is being increased as result of adrenalin

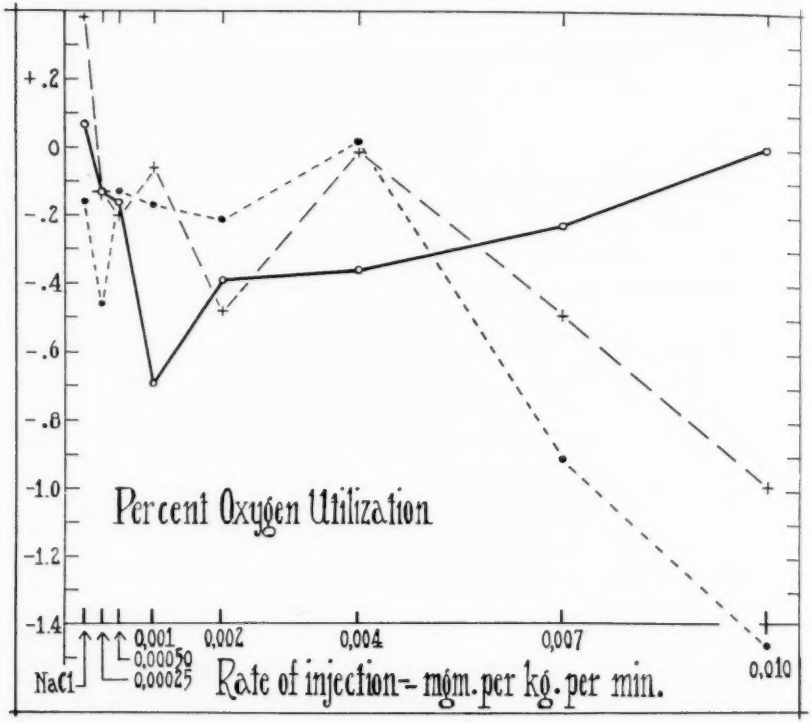

Fig. 3. The percentage oxygen utilization or the oxygen deficit of the expired air following 5-minute injection of isotonic NaCl and of adrenalin as described for figure 1.

The three curves have the same significance as the three curves of part I, figure 1 and of figure 2.

injection beyond immediate oxidative requirement to an extent not only preventing decrease in oxygen percentage (or increase in percentage utilization) of expired air, but enough actually to increase it (or decrease the deficit). The only probable cause, in terms of our data at least, of such over-ventilation would be the developing lactic-acid acidosis; which, also in part, would be responsible by addition of non-oxidative carbon dioxide

for failure of the over ventilation to lower the carbon dioxide percentage of the expired air.

It may be noted in confirmation of at least part of the above mechanism that maximum increase in oxygen percentage (fig. 2; or decrease of oxygen deficit, fig. 3) coincides with maximum pulmonary ventilation as produced by injection at the rate of 0.001 mgm. per kilo per minute; and that the effects parallel each other with great fidelity at each of the higher rates of administration. Also the progressive increase in the percentage of carbon dioxide in the expired air up to injection at the rate of 0.002 mgm. per kilo per minute seems satisfactorily explained by considering it a resultant of metabolic production, lactic acid displacement and ventilation, as integrated above.

Further application of this reasoning would seem, however, to lead to expectation of continued increase in carbon dioxide content of the expired air as: 1, blood lactic acid increases; 2, metabolic rate, as measured by oxygen consumption, is undiminished (two previous reports); and 3, pulmonary ventilation continues to decrease, with the three highest rates of injection. The only unknown which might vary so as to produce the observed slight decrease in carbon dioxide concentration of the expired air under these conditions, at rates of injection above 0.002 mgm. per kilo per minute, would seem to be its rate of metabolic formation. A decrease in this, such as might result from a shift from carbohydrate to fat metabolism would seem to be the only way in which to explain a decrease in the percentage of carbon dioxide in the expired air coincident with uniform oxygen intake, diminished pulmonary ventilation and augmented blood lactic acid. While hesitating to advance this seemingly unsupported and *post hoc* explanation, it may be recalled that evidence of quite a different nature in favor of increased metabolism of fat under action of adrenalin has previously been advanced by the Coris (1928); so its use to explain the present results is not too unwarranted.

2. *Pulmonary ventilation 5–10 minutes following injection* (fig. 1, I, dashed line): interpretation of changes in pulmonary ventilation this length of time after injection must proceed without invocation of blood pressure effects, since according to the little information available this returns to normal within a minute or less of cessation of injection. Metabolic rate and blood lactic acid concentration are thus left as the active controls to be considered.

Presumably the combined effect of these as stimulus to respiration is less at this time than during the injection, at rates of 0.001 and 0.002 mgm. per kilo per minute, since pulmonary ventilation shows a decline from the high levels reached during the injections themselves. It is still great enough, however, to maintain the oxygen percentage of the expired air (fig. 2) above, or the oxygen deficit (fig. 3) below, normal; though not so

great as to prevent slight retention of carbon dioxide (fig. 2), presumably in compensation for the excessive "auspumpung" during injection.

On the other hand it will be seen that at this interval after the injection, when this was at rates of 0.004, 0.007 and 0.010 mgm. per kilo per minute, pulmonary ventilation shows a phenomenon very characteristic of the effects previously described, viz., continued increase after injection is stopped. Following 0.004 mgm. per kilo per minute this could be due to the combined effect of metabolic rate and blood lactic acid; but is probably chiefly conditioned by the former, since oxygen consumption reaches its greatest increase for the entire series (+16 per cent) at this time, i.e., 5 to 10 minutes after injection at this rate. Also in favor of metabolic rate being chiefly in control at this time after this rate of injection is the fact that composition of expired air (figs. 2 and 3) shows less abnormality than after any other effective rate of injection.

This last is in sharp contrast to the effects following injection at the two highest rates; pulmonary ventilation at this 5 to 10 minute interval after injection is progressively increased and out of all proportion to metabolic need: as evidenced by the fact previously adduced that oxygen consumption is actually depressed by these rates of injection (previous report); and as shown by the reciprocally related changes in composition of the expired air (figs. 2 and 3). The persistently augmenting blood lactic acid (previous report) must be alone responsible for these effects following these two highest rates of injection.

3. *Pulmonary ventilation 25 to 30 minutes after injection* (fig. 1, I, dotted line): it is unnecessary to apply the above analysis to every detail of this curve. At this time interval following injection at rates of 0.001, 0.002 and 0.004 mgm. per kilo per minute, either metabolic rate or blood lactic acid or both (previous reports) have diminished sufficiently to explain the return to nearly normal of the pulmonary minute-volume. And again, the continuing increase to the end of this half-hour post-injection period following administration of 0.007 and 0.010 mgm. per kilo per minute is the reverse of metabolic rate at this time but directly parallel with change in blood lactic acid concentration and, therefore, presumably due entirely to it; an interpretation in harmony, likewise, with the changes in composition of the expired air (fig. 2 and 3).

4. *Total, summated effect for the 35-minute experimental period* (fig. 1, II): the only additional contribution afforded by the evidence expressed in this curve is the clear demonstration of the complete dissimilarity between the total calorigenic effect of adrenalin and its effect on the volume of pulmonary ventilation. As shown in one of the preceding reports, total oxygen consumption for this 35-minute period rises uniformly from threshold to a maximum when injection was at the rate of 0.004 mgm. per kilo per minute; then declines just as uniformly as rate of injection is increased beyond this. Pulmonary ventilation does not parallel this even approxi-

mately even at the lower rates of injection; and at the higher ones, is the direct antithesis of it. Whether the attempted explanation of this lack of correspondence in terms of known or postulated coincident changes in blood pressure and blood lactic acid concentration is significant or not, recognition of this diversity is fundamental to proper interpretation of measurements of the respiratory metabolism following adrenalin injection. This will receive additional consideration later in the attempt to evaluate changes in carbon dioxide output and respiratory quotient observed in these experiments.

COMMENT. It seems unnecessary to burden this short report with a detailed review of the very large literature dealing with the effect of adrenalin on respiration; especially since, insofar as we have been able to find, there is nothing in it very closely similar to what is being reported here. In brief summary, preceding work divides almost completely into two sharply separated categories. The bulk of it deals with the immediate effect in acute animal experiments of brief intravenous injections of adrenalin in amounts usually unspecified or which when given prove to be physiologically enormous and beyond any concentrations employed in this work. Even Nice, Rock and Courtright (1914) who seem to be the only ones to give deliberate attention to the possible differential effect of dosage, employed for their smallest, concentrations apparently four times our maximum. These they found stimulated respiration and thus provided explanation for this type of effect as occasionally reported by their predecessors. Large doses on the other hand produced the depression of respiration or adrenalin apnea which has usually been reported for this type of experiment. The mechanism of this reaction is apparently still imperfectly explained in minor details but seems in large part the result of reflex inhibition of the center from stimulation of the pressure-sensitive areas of the aorta and carotid sinus (Heymans and Bouckaert, 1930; Schmidt, 1932). And this conception has been employed above in partial explanation of the results obtained by us during the 5-minute injection periods when hypertension would be a concomitant factor in the response.

On the other hand those who have primarily been interested in the calorigenic response to adrenalin have employed it with therapeutic caution, using either subcutaneous administration with its slow and delayed absorption, or more rarely, intravenous injection at supposedly physiological rates; and have apparently without exception observed a stimulation of respiration over metabolically significant periods which manifested itself by increase either of rate or depth or both. The apparent difficulty here has been properly to relate the calorigenic and respiratory effects so as to obtain explanation of the increase in respiration on the one hand and a metabolically significant interpretation of the respiratory exchange on the other. The data presented in this and the preceding reports of this series were collected with this aim primarily in view.

SUMMARY

Intravenous injection of adrenalin at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute for five minutes increases minute-volume of pulmonary ventilation of chloralose-anesthetized cats as follows:

1. The minimal effective rate of injection is 0.001 mgm. per kilo per minute; this is the same threshold as for blood lactic acid increase and for definite, sustained increase in oxygen consumption.

2. For the 5-minute injection period maximum increase in pulmonary ventilation occurs also at threshold rate of administration; at all higher rates of injection the increase during injection itself is progressively less; this is altogether unlike the effect shown either by oxygen intake or blood lactic acid and is attributed to the inhibitory effect of the concomitant hypertension which very probably is directly proportional to dosage.

3. Following administration at rates of 0.001 and 0.002 mgm. per kilo per minute ventilation reaches its maximum increase during injection and then returns nearly to normal by the end of another 20 minutes (i.e., half-an-hour after injection). This is very similar to the effect on oxygen consumption and tentatively may be regarded as adaptation to metabolic need.

Following administration at rates of 0.007 and 0.010 mgm. per kilo per minute increase in ventilation continues progressively to the end of the subsequent 30-minute interval which was as long as the effect was followed in this work. This is similar to the lactacidemia following these two maximal rates of injection and may be ascribed to it.

These interpretations are confirmed by the changes which are observed in composition of the expired air.

These results emphasize the complex nature of the respiratory response to adrenalin and by just so much the caution necessary in interpretation of determinations of the respiratory metabolism under the action of this hormone.

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A COMPARISON OF SOME RESPIRATORY AND CIRCULATORY REACTIONS OF ATHLETES AND NON-ATHLETES[1]

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Comparisons are here given of 4 athletes under training with 4 men who at the time confined their physical activity to the routine of a laboratory. The condition of the subjects was as follows: 3 of the untrained men (see table 1, W. O., J. H. and M. B.) had taken almost no exercise during the college year and prior to that had never undergone regular athletic training; while S. R. (table 1), also untrained, had in former years been a member of a swimming team but had not within a year trained for the sport. The athletes were under observation while in training and were in excellent physical condition. S. S. was a swimmer, R. T. a basketball player, and L. B. and G. B. distance runners.

The data assembled in table 1 were prepared from the last of the experiments on each subject. Each record as given is substantially the same as for earlier experiments with the individual.

RESPIRATORY DIFFERENCES. According to Hörnicke (4) training does not alter the respiratory minute volume during rest. Schneider and Ring (7), among others, found that trained men breathe less air for the same accomplishment than untrained men. The differences brought about by training are not so clearly demonstrated in a comparison of groups as when the changes occurring in an individual are followed step by step. Schneider and Ring in their study of 2 subjects found that the breathing became more economical with the passing of the weeks; that is, the minute-volume of breathing for any given load of work decreased steadily with training and reached its lowest level in from 4 to 6 weeks.

In the present investigation the respiratory minute-volume during rest averaged practically the same for the men both in and out of training. However, when their breathing was calculated in terms of body surface, a moderate difference was evident. The untrained group then averaged 5.21 L. and the trained group 4.78 L. per square meter of surface. During work, 6000 ft. lbs. per minute, some difference was also observed in the minute-volume of air breathed; the untrained group averaged 42.4 and

[1] This study was made possible by a grant from the Denison Foundation for Biological Research at Wesleyan University.

the trained group 40.1 L. The difference is further emphasized when averaged in terms of body surface; namely, 23.5 and 20.4 L. respectively.

TABLE 1

SUBJECT	BODY WEIGHT	OXYGEN CONSUMPTION	O_2 CONSUMPTION PER SQ. M. SKIN SURFACE	VENTILATION EQUIVALENT	PULSE RATE	O_2 PULSE	CARDIAC OUTPUT	CARDIAC OUTPUT PER SQ. M. SURFACE AREA	ARTERIO-VENOUS DIFFERENCE, PER LITER OF BLOOD	SYSTOLIC OUTPUT PER BEAT
				At rest on the bicycle ergometer						
	kgm.	*cc. per min.*	*cc.*	*liters*		*cc.*	*liters per min.*	*liters*	*cc.*	*cc.*
Non-athlete:										
W. O.	69	330	169	3.2	80	4.1	4.5	2.3	74	56
J. H.	61	335	193	3.8	89	3.8	5.3	3.0	62	64
M. B.	73	305	163	2.5	82	3.7	3.6	1.9	84	44
S. R.	63	265	172	2.7	78	3.4	4.4	2.6	61	56
Average		309	174	3.1	82	3.8	4.5	2.5	70	55
Athlete:										
S. S.	75	305	156	2.9	56	5.4	6.1	3.1	62	93
R. T.	88	375	173	2.9	71	5.3	4.4	2.0	86	62
L. B.	68	340	181	2.5	61	5.6	4.5	2.4	76	74
G. B.	65	300	166	3.2	55	5.5	5.2	2.9	58	95
Average		330	169	2.9	61	5.5	5.1	2.6	71	81
				At work, 6000 ft. lbs. per minute						
Non-athlete:										
W. O.		1680	862	2.3	144	11.7	17.7	9.1	95	123
J. H.		1780	1022	2.5	168	10.6	16.8	9.7	106	99
M. B.		1760	942	2.5	156	11.3	14.7	7.9	120	95
S. R.		1830	1078	2.3	150	12.2	14.7	8.7	124	98
Average		1763	976	2.4	155	11.5	16.0	8.9	111	104
Athlete:										
S. S.		1830	940	2.0	124	14.8	16.1	8.3	114	130
R. T.		2250	1038	2.4	140	16.1	18.7	8.6	120	134
L. B.		1930	1028	1.8	132	14.6	19.5	10.4	99	148
G. B.		1720	951	1.9	118	14.5	17.0	9.4	101	144
Average		1933	989	2.0	129	15.0	17.8	9.2	109	139

The gain in trained men in economy of breathing is also brought out by the ventilation equivalent (see table 1), defined as the volume of air which has to be inspired for each 100 cc. of oxygen absorbed. During rest the

ventilation equivalent of each of the 2 groups was only slightly different; i.e., 3.1 for the untrained and 2.9 L. for the trained; but during work this was clearly reduced, the average being 2.4 for the former and only 2 L. for the latter group.

Data for the consumption of oxygen (see table 1) when considered without any correction justify the opinion that the athlete consumes more oxygen than the non-athlete both during rest and work. When, however, the intake per square meter of body surface is considered little, if any, difference is found between the 2 groups. Thus the average amount of oxygen consumed per square meter of skin surface was 174 cc. for the untrained and 169 cc. for the trained while at rest, and 976 cc. for the untrained and 989 cc. for the trained men with 6000 ft. lbs. of work. Had the load been excessive for the non-athlete there is every reason to believe that his intake of oxygen would then have been much less than that of the athlete. The withdrawal of oxygen from a liter of blood, as shown by the arterio-venous difference, averaged practically the same for both groups of men. During rest the average for the athlete was 71 cc. and for the non-athlete 70 cc., while during work it was 109 and 111 cc. respectively.

CIRCULATORY DIFFERENCES. It has been evident for some time that training lowers the pulse rate, increases the circulatory minute-volume, and increases the output of the heart per beat.

Pulse rate. The difference in the physical fitness of our 2 groups is indicated by the pulse rate as the men sat at rest on the bicycle ergometer. The rate averaged 82 beats per minute for the untrained and only 61 for the trained men. A similar difference was observed during the period of work, when the frequency averaged 155 and 129 respectively. While the heart of the trained man during the work period had the advantage of starting at a slower rate than that of the untrained, his percentage rise exceeded that of the untrained man. The average acceleration for the former was 111 per cent as compared with 89 per cent for the latter group. It is noteworthy, however, that while carrying the load of 6000 ft. lbs. the trained men still retained a large part of their margin of response; while the frequency of the heart beat of the untrained men was already nearing the rate (160–180) at which the period of diastole becomes too brief to allow the ventricles to fill completely, and at which the mechanical efficiency of the heart begins to decrease.

Oxygen pulse. This has been defined as the amount of oxygen consumed by the body from the blood of one systolic discharge of the heart. It is calculated by dividing the amount of oxygen absorbed per minute by the number of heart beats in a minute. It is believed that the size of the oxygen pulse gives an index of the output of the heart per beat. If this interpretation be accepted, it is evident that the untrained men had

during rest a smaller systolic discharge than the trained men. The average oxygen pulse for the untrained men was 3.8 cc. and for the trained men 5.5 cc.

During the effort of 6000 ft. lbs. per minute the oxygen pulse of the untrained men ranged between 10.6 and 12.2 cc., average 11.5 cc.; while in the trained men it ranged from 14.5 to 16.1 cc., average 15 cc. This indicates a smaller stroke volume of the heart in the untrained men both when at rest and at work than obtains among trained individuals.

When the oxygen pulse is calculated for the resting state in terms of skin area, a rather marked uniformity is indicated within each of our 2 groups of men. For the 4 untrained men this index was 2.1, 2.2, 2.0, and 2.0 respectively; and for the trained men 2.8, 2.4, 3.0, and 3.0 respectively. The systolic volume is thought to be more definitely related to body weight than skin area, therefore we have calculated the relationship between the oxygen pulse and body weight. The index so obtained, i.e., oxygen pulse divided by body weight, does not differentiate as clearly between untrained and trained men. The values obtained for the 4 untrained men were 0.06, 0.062, 0.051 and 0.054 respectively; and for the trained men, 0.072, 0.060, 0.082 and 0.085 respectively. According to this index one of the men in training should have been classified with the untrained group.

Output of the heart. For these determinations Christensen's (2) modification of Grollman's acetylene method was used. The samples of the respired mixture were taken during rest at the 15th and 22nd seconds and during work at the 6th and 10th seconds of rebreathing. Consistent and uniform results in repeated experiments lead to the opinion that the data give a true picture of the volume of blood that was discharged.

The amount of blood pumped by each group of men was not different as they were seated at rest on the bicycle ergometer. The average output per minute was 4.5 liters for the untrained and 5.1 liters for the trained group. This difference was due to a low output by M. B. (see table 1) among the untrained group and a large output by S. S. among the trained men. The other 3 members of the two groups match almost exactly. The values for minute-volume of blood pumped per square meter of body surface are clearly equal, with an average value of 2.5 L. for the untrained and 2.6 L. for the trained men.

These results are not in accord with Lindhard's (6) who, with the nitrous oxide method, found in the resting athlete a larger minute-volume than in the non-athlete. Henderson, Haggard and Dolley (3) by the ethyl iodide method found very little, if any, difference in the resting circulation of athletes and non-athletes.

Even during work we find very little difference in the minute-volume of our 2 groups. It is true that when the data for the volume of circulation

are considered without regard to weight and body surface the athletes have, on the average, the larger minute-volume. When, however, the circulation is calculated in terms of body surface there is little or no difference in the 2 groups. The average minute-volume during exertion for the non-athletes is 8.9 L. and for the athletes 9.2 L. per square meter of body surface. An inspection, in table 1, of the individual cases fails to show a uniform difference between the 2 groups. Hence we may conclude that if the 2 groups are representative of the non-athlete and athlete, the minute-volume of blood pumped per minute both during rest and exertion is not necessarily larger among athletes than non-athletes. This of course can be true only when the load of work is normal; that is, one in which the organism is able to supply the amount of oxygen needed by the active tissues of the body. Had a 6000 ft. lb. load of work been an overload for the non-athletes a difference in the minute-volume of the 2 groups would have occurred because of the ability of the athlete through training to provide more oxygen.

STROKE VOLUME OF THE HEART. The output of the heart per beat was during rest clearly less among non-athletes. This holds for both the individual and group averages. Among the non-athletes the stroke volume ranged from 44 to 64 cc., average 53 cc.; and among the athletes from 62 to 95 cc., average 81 cc. Likewise during the exertion with a load of 6000 ft. lbs. the stroke volume of the non-athlete was consistently less than that of the athlete. The range for the former was 95 to 123 cc., average 104; and for the latter 130 to 148 cc., average 139 cc. Both groups compensated during exertion by increasing the stroke volume; the non-athletes by an average of 51 cc. and the athletes by 58 cc.

Our findings differ from those of Henderson, Haggard, and Dolley (3) who reported that in non-athletes the stroke of the heart is of approximately the same volume during rest and exercise, although in athletes during exertion the stroke volume increases as much as 50 per cent. Bock and co-workers (1) working with athletes and non-athletes found that both experienced an increase. Our findings, therefore, support those of Bock and co-workers.

SUMMARY

When the minute-volume of breathing is stated in terms of square meters of skin surface or in terms of the ventilation equivalent the economy of the breathing of the athlete is emphasized.

The athlete and non-athlete, during rest and moderate exertion, consume approximately equal amounts of oxygen per square meter of body surface. They also withdraw equal amounts of oxygen from a liter of blood.

The clearest circulatory gains that result from a period of physical

training are a reduction in the frequency of heart beat and an increase in the output of the heart per beat. The minute-volume of circulation, when stated in terms of skin surface, seems to be approximately the same for athletes and non-athletes.

The oxygen pulse, when stated in terms of skin surface, gives a good index of differences in heart stroke as found for the athlete and non-athlete.

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INJURY TO THE ILEAL MUCOSA BY CONTACT WITH DISTILLED WATER

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In a series of papers, Visscher, Ingraham, and Burns (1) (5) (6) (7) have shown that the ileum, especially the lower portion, is capable of doing osmotic work in absorption. Their procedure was to make acute experiments on amytal anesthetized dogs, to wash out the content of the segment to be used with large amounts of 0.9 per cent NaCl solution, and to insert a solution half isotonic in NaCl and half isotonic in Na_2SO_4, all fluids being introduced at body temperature and low pressures. Under these conditions they found that the chloride was almost completely removed from the lumen in 60 to 90 minutes, the sulfate was sometimes almost completely retained in the lumen, and water disappeared in such amounts that the osmotic pressure of the loop fluid remained about equal to that of the plasma. In other words, the chloride was impoverished in the bowel and the sulfate was concentrated to about twice the original value.

In some experiments on these phenomena on the chronic isolated gut loop (Thiry-Vella) without anesthesia, some interesting effects of distilled water were observed. In general the technique was as described elsewhere (2) (3).

EXPERIMENTAL FINDINGS. In the course of an experiment, the dog lay quietly while the loop was washed, the test fluid inserted, and the experiment run (3). For several weeks the washing necessary to remove mucus and debris prior to each experiment was made with distilled water in the hope that the lumen could be made entirely chloride-free before the introduction of the test fluid. Under these circumstances it was found impossible to corroborate the findings of the acute experiments, for the bowel acted like a permeable membrane, the chloride concentration falling very little, sulfate diffusing rapidly out of the lumen, and water being absorbed only slowly, as shown in figure 1.

When preliminary washing was performed with 0.9 per cent NaCl instead of distilled water, the bowel functioned as Visscher and his co-workers had found for acute experiments under anesthesia, with concentration of sulfate, rapid removal of chloride, and rapid absorption of water (fig. 1).

Burns and Visscher (1) found in acute experiments that if distilled water

was left in the bowel, the concentration of chloride rose to a level higher than that of an ultrafiltrate of plasma. After the demonstration of injury by distilled water, it now seemed likely that these changes in chloride concentration might have been due to such injury. To test this possibility, 50 cc. of distilled water were placed in a 60 cm. low ileal loop in a waking dog and the chloride concentration was followed; it rose to 92 millimols per liter in 30 minutes. After allowing several days for recovery, a solution isotonic in sucrose was introduced into the same loop under the same conditions, and the chloride concentration rose only to 15 millimols per

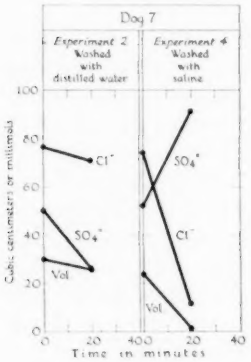


Fig. 1

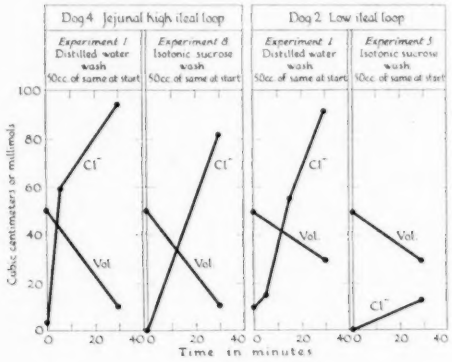


Fig. 2

Fig. 1. Interference with chloride impoverishment and sulfate concentration occasioned by preliminary washing of a chronic ileal loop with distilled water. Concentration changes were normal in experiment 4, several days later, and after 0.9 per cent NaCl preliminary washing. The test fluid inserted in each case was one half isotonic in NaCl and one half isotonic in Na_2SO_4.

Fig. 2. *Dog 4.* Graph illustrating rapid rise in chloride concentration in contents of a chronic jejunal loop whether isotonic sucrose or distilled water was inserted. *Dog 2.* Graph illustrating rapid rise in chloride concentration in chronic low ileal loop contents after insertion of distilled water, and slow rise at a later date in the same loop after insertion of isotonic sucrose. (Blood level 100 mM/L.)

liter in 30 minutes. The volume changes were practically identical in the two experiments (fig. 2, dog 2). Inasmuch as the presence of sucrose could not interfere with the simple diffusion of chloride, the conclusion seems inescapable that the mechanism underlying the difference is mucosal injury.

A similar pair of experiments was done on a jejunal-high ileal loop. Here the difference in chloride accumulation under the two conditions is insignificant (fig. 2, dog 4). This does not necessarily indicate absence of injury, for the secretion of this portion of the bowel normally contains much chloride.

It was also observed that if active chloride improverishment were interrupted by withdrawal of the gut fluid and washing quickly with 200 to 500 cc. of distilled water, the amount of chloride in these distilled water washings exceeded by as much as 600 per cent (average—200 per cent in 7 experiments on 2 dogs) the amount of chloride expected on the basis of analysis of the withdrawn gut fluid for chloride, and estimation of the unaspiratable residual volume of this gut fluid based on the volume of the wash fluid and sulfate determinations on gut fluid and wash fluid. It might be argued that these differences indicate passage of sulfate out of the loop rather than of chloride into it, but in either case abnormal function of the gut is evident.

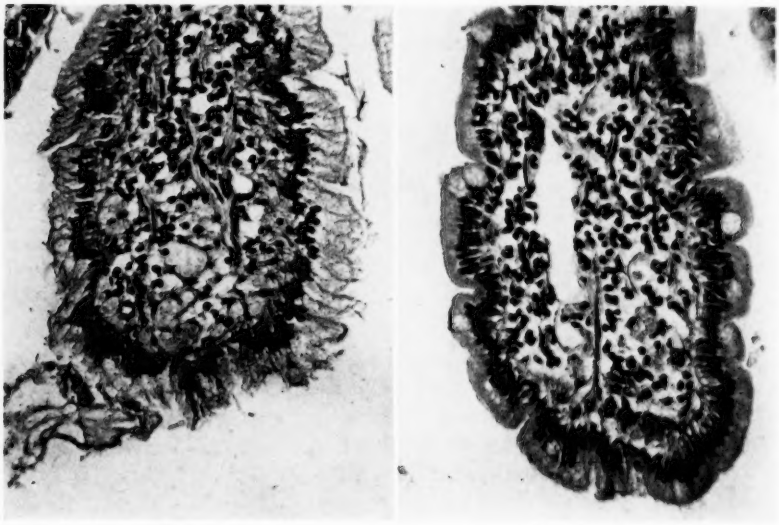
Three dogs were used for microscopic observation of the living mucous membrane. After anesthetization with intraperitoneal pentobarbital sodium (35 mgm. per kgm. of body weight), a short segment of ileum was delivered through a flank wound and opened by a longitudinal incision near the antimesenteric border. The mucosa was examined with a binocular dissecting microscope (80×) using alternately transillumination and indirect lighting. In a large number of observations of ileum and also jejunum, it was observed that strong villus movement persisted, together with normal morphology, whether the mucosa was washed with isotonic sodium chloride solution or left untouched in a humid atmosphere. Washing with distilled water resulted most commonly in an increase in villus activity for a few seconds, and then cessation of pumping action, occasionally replaced temporarily by waving motions of the villi. Within a minute or two after the washing with distilled water, the epithelial layer, which could usually be definitely discerned, was observed to swell to twice the original thickness, and at the same time the surface became irregular and frayed, horizontal clefts appearing which in places opened into the lumen, with peeling of the surface layer. When the area was washed with isotonic sodium chloride solution after distilled water washing, a fine film of grayish color appeared on the surface. This was visible to the naked eye, and could be peeled off with a needle, but resisted washing.

Similar studies with the colon showed no swelling, but some membrane formation.

In order to show this effect in fixed histological material two dogs were anesthetized with pentobarbital, and two adjacent segments at each level of the bowel were washed without interrupting the blood supply, one with a liter of isotonic sodium chloride, the other with distilled water, both at 38°C. Immediately after such washing in each segment, a short piece was dropped into 4 per cent formaldehyde, and hematoxylin and eosin-stained sections were prepared.

Examination of these fixed sections showed no evidence of damage in stomach or duodenum, and only minimal if any changes in the colon. In

the jejunum and ileum, however, changes were observed similar to those seen in the living tissue, but less marked. There was thickening of the epithelial cells near the ends of the villi where contact with the distilled water had been most intimate, while deeper in the crypts and on most of the sides of the villi these cells still looked normal. There was also more frequent fraying of the surface layer than in the saline-washed sections. The thin veil was occasionally to be seen draped from villus to villus and evidently consisted of coagulated material lost from the villus tips on fraying of the surface (fig. 3).



a (dist. water) b (saline)

Fig. 3. Comparison of the effects of isotonic sodium chloride solution and distilled water on the epithelium of the dog ileum. In each case the segment, with blood supply intact, was washed with 1000 cc. of the fluid in question and immediately dropped into 4 per cent formaldehyde solution. Hematoxylin and eosin stain. Magnification 300×.

Comment. The finding of this injury prompted a search through the literature. E. Waymouth Reid (9) observed in 1898 that in portions of the small intestine the epithelium is very susceptible to strength of solutions employed and especially to distilled water. He reported that the mere washing of a loop of small gut with distilled water was followed by less absorption from the animal's own serum than occurred in a control washed with 0.9 per cent NaCl. Goldschmidt and Dayton (4) differed with Reid on this point, evidently overlooking the possibility that the

colon may be more resistant than the ileum; they found that in the colon, chloride impoverishment occurred even immediately after washing with distilled water. Magee and Reid (8) observed that the mucosa and gut wall were injured by distilled water, after which phosphate diffused 100 per cent faster than through the normal intestinal wall.

SUMMARY AND CONCLUSIONS

Injury to the mucosa of the lower ileum by contact with distilled water has been demonstrated by interference with the impermeability of this membrane to sulfate and by interference with the ability of the epithelium to do osmotic work. Injury of the mucosa of the entire jejunum and ileum by distilled water has been demonstrated microscopically. The implications of these observations are evident. Certain published studies concerning the activity of the gut have been mentioned which need reconsideration. In addition, a practical application of the findings discussed in this paper is found in criticism of the use of distilled water in clinical practice for irrigation of the small intestine. The mucosa of this part of the alimentary tract is never normally exposed to strongly hypotonic solutions, and their use under artificial conditions is not justified. It is suggested that some isotonic solution such as 0.9 per cent NaCl be used instead of distilled water for irrigations of the small intestine in clinical cases of bowel obstruction.

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THE INFLUENCE OF VARIOUS FACTORS UPON INTESTINAL ABSORPTION INVOLVING OSMOTIC WORK IN THE UNANESTHETIZED DOG

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In a series of studies (1, 4, 5) it has been demonstrated that the lower ileum of the anesthetized dog does osmotic work in absorbing NaCl against a concentration gradient from solutions containing mixtures of NaCl and Na_2SO_4. It seemed important to determine whether similar phenomena could be observed in the unanesthetized dog with a Thiry-Vella loop (7). It was anticipated that certain processes might proceed at different rates in the presence and in the absence of anesthesia.

MATERIAL. Thiry-Vella loops of 20 to 60 cm. length were prepared on 10 dogs by a technique described elsewhere (2). In 8 animals the loops were at the level of the lower ileum, in one the midportion of the ileum, and in one the jejunum. Forty-six experiments on these 10 animals comprise the basis for this report.

TECHNIQUE. The stomata were made in the left flank, and the dogs were trained to lie with that side up. Conical plugs were made of sponge rubber covered with rubber dam to close the stomata during experiments. For purposes of withdrawal of fluid a number 10 French soft rubber catheter with multiple holes had been inserted through the upper plug so as to extend about 20 cm. into the loop, thus reaching about the middle of the loop.

The loops were washed with isotonic sodium chloride at body temperature prior to each experiment, and ten to twenty minutes' time was allowed to pass for the absorption of residual fluid and salt. The test fluid then introduced at body temperature was half-isotonic in sodium chloride and half-isotonic in sodium sulfate, small enough volumes being used not to cause the animal to give evidence of discomfort, usually 15 to 40 cc. A few minutes later 8 cc. were removed and reintroduced through the catheter three times for mixing, using a cleaned and dried 10 cc. glass syringe, and either 1 or 2 cc. were taken for analysis to determine the composition at that time. The fluid in the catheter was forced back into the gut with 2 cc. of air. Additional samples were taken in precisely the same fashion at intervals until the difficulty in getting 8 cc. for mixing, and the increasing turbidity of the fluid removed, indicated that the loop was nearly

empty. In a number of experiments only an initial and a final analysis were made. The original volume was taken as the amount introduced, and the final was determined by withdrawal of as much as possible of the remaining fluid and then washing the loop with distilled water, in the earlier experiments, and with isotonic NaCl in the later ones, keeping the two portions separate. From analyses of both for sulfate the amount of fluid left in the loop was calculated and the volume so determined was added to the measured quantity withdrawn to give the true final volume.

Chloride was determined by the method of Van Slyke (6). Sulfate was determined by a modification of the benzidine method outlined by Visscher and Smith (8). The solution to be tested is freed of protein by addition of 1 part in 50 of glacial acetic acid. A volume of filtrate not to exceed 5 cc., and containing from 0.01 to 0.05 millimol of sulfate is placed in a 15 cc. conical centrifuge tube, and the total volume is made up to 10 cc. with a solution of benzidine hydrochloride prepared by solution of 4 grams of benzidine base and 5 cc. of concentrated HCl per liter in distilled water. The benzidine sulfate precipitate is centrifuged down at 2600 revolutions per minute for 15 minutes, the supernatant fluid is poured off, and the inverted tube allowed to drain 3 minutes. The precipitate is washed with 5 cc. of saturated benzidine sulfate solution, the stirring rod being washed down with 3 cc. more of the same, and the tube is re-centrifuged 15 minutes. A second washing, centrifuging, and drainage is followed by addition of 2 to 3 cc. of water and a drop of phenolphthalein indicator and titration at 80 to 90 degrees C. with 0.02 N NaOH until the pink color just persists (a mechanical stirring device simplifies titration). This titration theoretically follows a stoicheiometric relation, but actually varies somewhat due to slight solubility of benzidine sulfate, excess of NaOH needed for color reaction, etc., and therefore most accurate results are obtained by plotting mM SO_4'' against cubic centimeter NaOH to provide an empirical standardization. The dilute alkali must be kept from the air in paraffin-lined metal containers. The method is accurate within $\pm$ 2 per cent as here described.

Results. I. *Uni-univalent salt impoverishment in the unanesthetized dog.* The analytical results of typical experiments using loops at three levels of the small intestine are shown in figure 1. It will be seen that the high jejunal loop shows no chloride impoverishment, but instead the concentration of that ion in the gut fluid rises to the blood plasma level (about 100 mM per liter) and the sulfate concentration falls. In the middle and lower ileal loops, on the other hand, the chloride concentration falls rapidly, while the sulfate increases, these changes being fastest in the terminal ileal segment. These observations on the behavior of the small intestine at the several levels are in confirmation of previous results on anesthetized animals (4).

Comparison of the rates at which chloride is cleared from the intestine

in acute and in chronic loop experiments shows that the process is faster in the latter (table 1). This finding and the fact, mentioned below, that in good chronic loop dogs, anesthetization does not appreciably alter the rate of absorption, suggest that the slower course of events in the acute experiments earlier reported (1, 4, 5) is due to the necessarily poorer

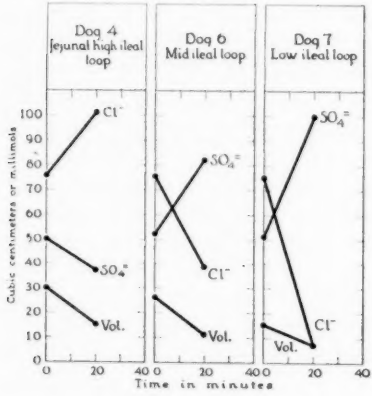

Fig. 1

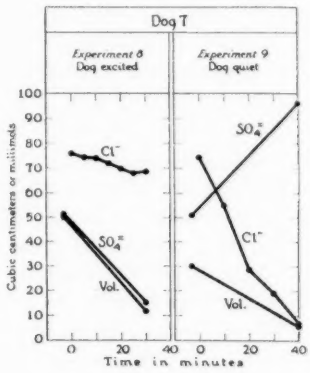

Fig. 2

Fig. 1. The behavior of segments of small intestine from three different levels toward an isotonic solution containing NaCl and Na_2SO_4.

Fig. 2. The effect of excitement on the behavior of the same chronic low ileal loop toward an isotonic solution containing NaCl and Na_2SO_4.

TABLE 1

Comparison of acute with chronic loop experiments with regard to the time required to lower the chloride concentration from 77 to 20 mM per liter

	NUMBER OF EXPERIMENTS	MEAN TIME	MAXIMUM TIME	MINIMUM TIME
		min.	*min.*	*min.*
Acute*	24	46	95	25
Chronic loop	13	18	36	8

* Experiments previously published (1, 4, 5).

condition of the animal due to incipient shock, and to the handling of the intestine itself.

II. *The influence of excitement upon intestinal absorption.* Incidental observations pointed attention to the rôle that excitement might play in the factors controlling the rate of intestinal absorption. It was noted that those animals which would not be quiet on the observation table were apt to show lower rates of active absorption.

Experiments to test this point were of three types. In the first group (four dogs) comparative experiments were run, on the one hand in a quiet room with no known disturbing factors, and on the other hand with attempts to frighten or excite the animal. Some difficulty was encountered in arousing animals which had been adequately trained to lie quietly, and in spite of unusual noises, rocking the observation table, blowing an air stream at the animal's head, etc., none developed a widely dilated pupil or a pulse appreciably above 110 beats per minute, although efforts were made to get off the table. In these animals no significant differences in absorption rates of chloride from sulfate-containing solutions were observed. In one dog, however, pain evidently of acute type, was accidentally inflicted by overdistention of the loop at the start of the experiment, resulting in excitement which lasted several hours. In this case chloride ion was poorly absorbed, and sulfate diffused out of the gut (fig. 2, expt. 8), while under conditions of quiet at a later date in the same dog absorption was normal (fig. 2, expt. 9). It is possible that the overdistention itself was responsible for a part of this change.

In one dog an effort was made to duplicate the systemic effects of excitement by subcutaneous injection of 1.6 cc. of adrenalin hydrochloride, 1:1000, over the absorption period of 25 minutes. At first a great rise in pulse, with excitement, occurred, but this could not be maintained throughout; the rate of reduction of concentration of chloride ion during this experiment was 1.8 mM/L. per minute as compared to 2.3 mM/L. in the control experiment.

More definite results were obtained by the use of dogs which could not be satisfactorily trained, and remained tense and trembling on the table. In two such animals, one showed slight improvement in absorption upon administration of pentobarbital sodium intraperitoneally; the other showed very radical improvement (fig. 3). In two of the well-trained dogs used as controls, one showed no change in absorption whatsoever on administration of pentobarbital, and the other showed slightly less effective absorption. It is inferred that anesthesia removed the conditions which in the unanesthetized state in untrainable animals prevented rapid absorption. Identity of results with and without anesthesia is therefore an excellent test of the state of training of the dog.

Findings of the type just reported may have their explanation in the observation of Drury, Florey, and Florey (3), who noted that excitement states were associated with blanching of the mucosa of the exteriorized colon. Repetition of these observations upon exteriorized segments of small intestine in these studies has confirmed the above finding. Strong blanching still occurred upon excitement 3 months after exteriorization of the mucosa.

III. *The effect of feeding upon absorption from isolated loops.* Experi-

ments have been performed in which the same animals were studied before and after feeding a meal of mixed table scraps. The results of typical experiments are shown in figure 4. It will be seen that the differences are not marked. In fact they are well within the usual range of intra-individual variation in the dogs in question.

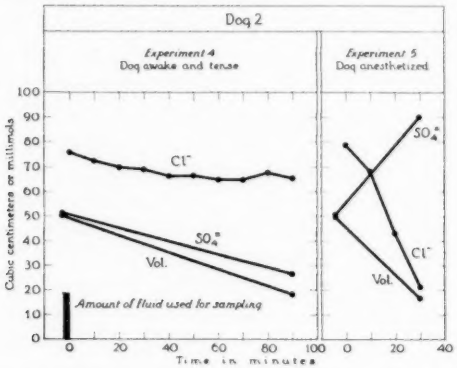

Fig. 3. The effect of light anesthesia upon the behavior of the same chronic low ileal loop in an excitable dog which could not be satisfactorily trained. The initial fluid in each case is half isotonic in NaCl and half isotonic in Na_2SO_4.

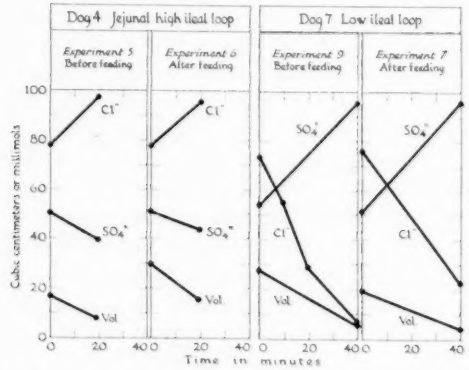

Fig. 4. Graphs of experiments illustrating the lack of effect of feeding on the behavior of either high or low ileal chronic loops toward an isotonic solution containing NaCl and Na_2SO_4.

SUMMARY AND CONCLUSIONS

1. Active absorption (against large concentration gradients) occurs from the lower ileum of the trained unanesthetized dog.

2. Thiry-Vella loops from the upper, middle, and lower ileum show progressively more rapid absorption, in the order named.

3. True excitement is associated with a diminished absorption rate. Anesthetization of excitable dogs increases the rate of active absorption; anesthetization of placid dogs does not cause such a change. Excitement causes blanching of the ileal mucosa.

4. The rate of active absorption is faster in chronic loop experiments on the dog than in acute experiments.

5. Ingestion of food does not alter absorption rates outside the limits of normal intra-individual variability.

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INTESTINAL ABSORPTION IN THE ADRENALECTOMIZED DOG

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Allers and Kendall and others (1, 2, 3) have recently shown that animals from which the adrenal glands have been removed may be maintained in good condition without the administration of adrenal cortex extract. They point out that this required the administration of a well-balanced diet except that eight to fifteen grams of sodium chloride must be given daily, and that the potassium intake must remain below two hundred milligrams per day. Administration of even small amounts of potassium above this level in the diet of adrenalectomized dogs caused toxic effects not seen in the normal animal, along with an elevation in the plasma potassium level.

The fundamental defect in metabolism resulting from withdrawal of cortical hormone might possibly be a decreased ability of the membranes of the body to maintain proper gradients of concentration of certain salts and a decreased ability to perform osmotic work. The findings of Harrison and Darrow (11) on changes in renal function resulting from withdrawal of the hormone led them to conclude that such was the case, for they found the rate of reabsorption of potassium in the renal tubules to be increased, and the rate of sodium reabsorption diminished, while the potassium in the urine could not be concentrated above the blood level more than a fourth as much as in control experiments.

Since the findings of Harrison and Darrow suggest that a general change in the characteristics of body membranes may result from withdrawal of the hormone, it seemed worth while to determine whether similar changes might be demonstrated in the intestine.

METHODS. The absorption rates of potassium, sodium, and chloride were studied by introducing solutions of these ions isotonic with the plasma into a segment of the terminal ileum, leaving them for measured periods of time, sometimes with the withdrawal of intermediate samples for study, and then evacuating all the fluid for analysis. Inasmuch as previous studies in this laboratory (7) (12) (13) (14) had shown that the presence of polyvalent ion salts hastens the fall in concentration of univalent ion salts, experiments were also performed in the presence of magnesium sulfate, the solution introduced always being isotonic with the plasma.

In the initial studies, acute experiments were performed upon the previously adrenalectomized dog, opening the abdomen, and handling the segment to be used. It was found that, despite essentially normal blood chemical findings, such procedures caused severe shock not seen in control animals. Subsequent experiments were therefore performed upon Thiry-Vella loops (18) in dogs trained to lie quietly on the table during experiments.

In this study, intestinal parasites were removed in all dogs by the use of tetrachlorethylene. The Thiry-Vella loops were prepared in healthy young dogs from forty-centimeter segments of lower ileum, the ends being tunnelled six centimeters under the skin over the last rib or spinal muscles in order that gentle pressure might effect complete closure of the stomata during absorption experiments (8).

Adrenalectomies were done in two stages by a dorsal, extraperitoneal approach. Following removal of the second gland, the dogs were regularly maintained on cortin (Wilson's Adrenal Cortex Extract) given parenterally for a few days and then placed on a high-sodium, low-potassium diet.[1]

In conducting experiments on trained dogs, the loop was washed gently with 0.92 per cent sodium chloride solution at 39 degrees centigrade, by means of a syringe and many-holed catheter inserted two-thirds the length of the segment; it was then emptied by aspiration, and the catheter was filled with air and left in place. Twenty minutes was allowed for absorption of residual wash fluid, a period based on determinations on rate of absorption of wash solution and volume not removable by aspiration. For the absorption period itself a fixed volume of either of the standard solutions[2] was inserted, and the loop was closed by pressure over the tunnelled portions and clamping of the air-filled catheter.

In some experiments repeated samples were taken, but ordinarily the loop was not disturbed for a given period of time; it was then aspirated to obtain the whole volume of fluid upon which the osmotic pressure and concentration of solutes were determined. Exact volumes in the loop were determined by adding to the volume aspirated a correction based in the sulfate-free experiments on comparison of chloride concentration in aspirated fluid with that in a measured amount of isotonic sucrose wash solution, but the sulfate ion was used for comparison when present because

[1] The authors are indebted to Dr. Mildred Ziegler and to Mr. William Stone of the Department of Pediatrics of the University of Minnesota Medical School for many suggestions concerning the care and feeding of adrenalectomized dogs.

[2] The standard KCl-NaCl solution contained 13.4 millimols of KCl per liter and 142.6 millimols of NaCl per liter. The standard KCl-NaCl-$MgSO_4$ solution contained 13.4 millimols of KCl per liter, 78.7 millimols of NaCl per liter, and 104 millimols of $MgSO_4$ per liter; it had a vapor pressure equivalent to a 159 millimolar solution of NaCl.

it offered more reliable results, the margin of error being regularly less than one-half cubic centimeter.

The dogs used for adrenalectomy were selected from a large number of chronic loop animals which had been studied for consistency of results obtained from day to day (8). Inasmuch as the large volume of fluid required for analyses prevented the routine withdrawal of samples mid-way in the absorption period, the course of absorption was followed by performing experiments of varying durations on the same animal.

In the acute experiments the abdomen was opened under intraperitoneal pentobarbital sodium anesthesia, the temperature of the animal being artificially maintained at 38°C., rectally. The ileum was gently washed with 3 liters of 0.92 per cent NaCl solution over a period of fifteen minutes, without removal from the abdomen, and the middle and lowest thirds were tied off into sections with a catheter in one end of each. The wound was closed with clips, the catheters emerging, and a forty minute recovery period was allowed. During the remainder of the experiment, fluid was handled through the catheters, without opening the wound, in much the same fashion as with the trained dogs.

After all experiments on adrenalectomized dogs, blood samples were drawn under oil, using heparin as anticoagulant, for determination of potassium, sodium, chloride, osmotic pressure, relative cell volume, and blood urea nitrogen. Osmotic pressure was determined by the method of Baldes[3] (4), chloride by the Van Slyke method (17), blood urea nitrogen by a modification of the Karr method[4] (15), potassium by a modification of the method of Breh and Gaebler (6), sodium by a modification of the method of Barber and Kolthoff (5), and sulfate by a modification of the benzidine method (9). Insofar as quantities allowed, all determinations were made in duplicate.

Results. Results are based on sixty-nine experiments in seven dogs, some acute, and some in Thiry-Vella loops, with more than half the experiments being controls.

Satisfactory experiments with regard to both control and post-adrenalectomy procedures were achieved in two dogs. Knowledge of the absolute amounts of potassium, sodium, and chloride at the start and at the finish of experiments of standard length allowed calculation of the number of millimols of each absorbed under conditions fixed except for the

[3] The authors wish to express their sincere appreciation to Dr. Raymond R. Roepke, Research Fellow in the Department of Physiology at the University of Minnesota, who set up the vapor pressure apparatus and himself did a large share of the determinations.

[4] The authors wish to express their thanks to Miss Beatrice Stevens, technician in the Experimental Laboratory of the Department of Surgery of the University of Minnesota Medical School, who made the blood urea nitrogen determinations for this study.

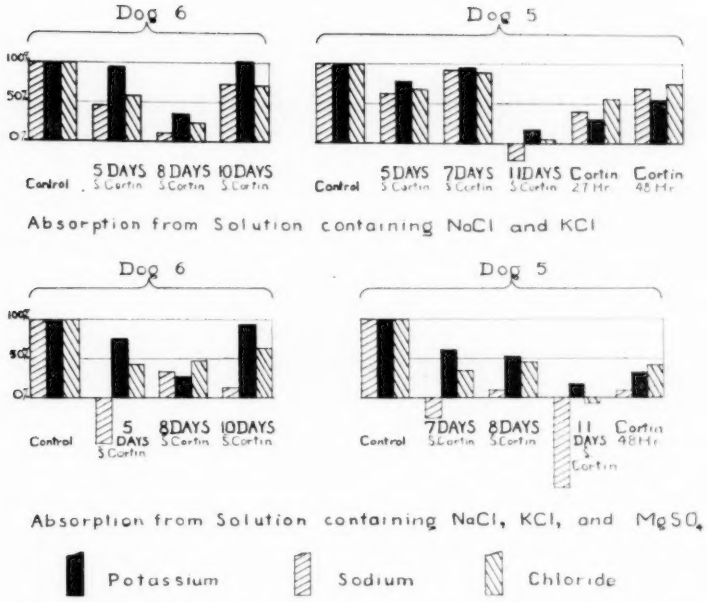

Fig. 1. Graphic presentation of effect of withdrawal of adrenal cortical hormone on absorption of $Na^{\cdot}$, $K^{\cdot}$, and Cl' from chronic ileal loops in trained dogs. The amounts of each ion absorbed in control experiments are used as a basis of comparison and evaluated 100 per cent for that purpose. The control amounts absorbed were:

	mM. absorbed		
	$Na^{\cdot}$	$K^{\cdot}$	Cl'
Dog 5			
From SO_4'' free solution	1.34	0.135	1.72
From SO_4'' containing solution	0.497	0.124	1.06
Dog 6			
From SO_4'' free solution	0.514	0.061	0.853
From SO_4'' containing solution	0.240	0.57	0.468

The blood chemical findings are presented in the following table.

	Dog 5					Dog 6		
	Days without cortin							
	5	7	8	11	Cortin treated 48 hours	5	8	10
Cell volume, per cent	42.3	43.8	47.1	52.2	38.9	40	39.7	38.4
Plasma Cl', mM. per liter	104	105	104	95	98	105	100	102
Plasma $Na^{\cdot}$, mM. per liter	142	146	137	131	137	147	142	140
Plasma $K^{\cdot}$, mM. per liter	4.7	5.3	4.9	6.0	4.7	3.5	4.8	5.2
B.U.N., mgm. per cent	14		16	19	16		12	16.6
Osmotic pr. in equiv. of mM. NaCl				154.32	150.74		158.77	150.63

state of the animal. For purposes of more ready comparison, presentation of these findings in figure 1 is in terms of per cent of the amounts of each ion absorbed in the control experiments. In both dogs, whether magnesium sulfate was present or not, the amount of each ion absorbed was decreased on withdrawal of cortical hormone, with the exception of potassium in one case, but the absorption of potassium was less delayed than that of sodium by a wide margin, with chloride usually falling be-

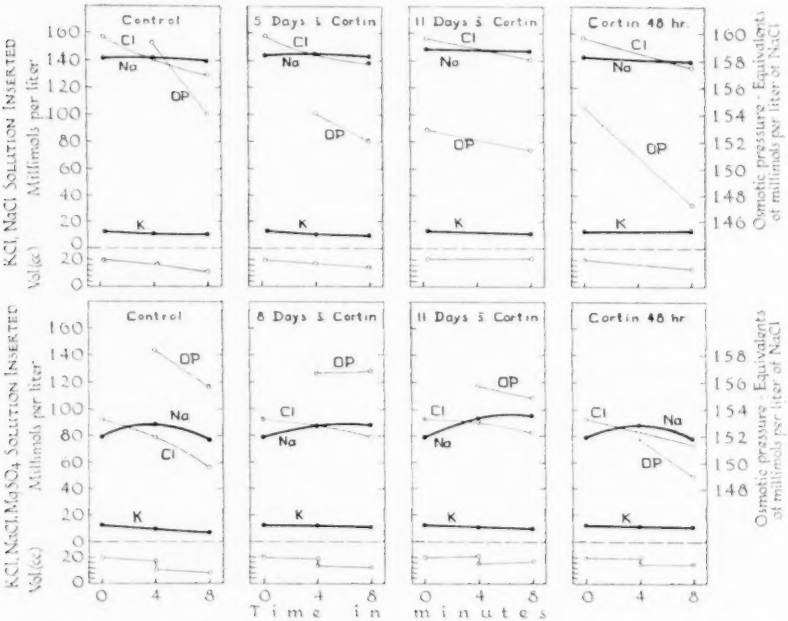

Fig. 2. Effect of withdrawal of adrenal cortical hormone on the curves of volume, osmotic pressure, and concentrations of the various ions in the solutions within the gut, dog 5. The effect of cortin treatment is also shown. Blood chemical findings are presented in the legend of figure 1.

tween. In four experiments, one in the absence of magnesium sulfate, there was an actual loss of sodium into the intestine, even though potassium was still being absorbed. In one experiment in dog 6, there was a slightly greater absorption of potassium after ten days without cortin than had been observed in the control experiments, but the sodium absorption was nevertheless diminished. These dogs deprived of cortin therefore absorbed not only a smaller amount of sodium than they had in control experiments, but a smaller amount relative to the quantity of potassium absorbed.

Administration of cortin to dog 5 in doses of 5 cc. twice daily after he

had been deprived of it for eleven days resulted in a change toward increased absorption of sodium, both absolutely and relative to potassium, and both in the presence and in the absence of magnesium sulfate.

The nature of the curves of volume and concentration change of the various ions during the absorption period altered after withdrawal of cortin as indicated by graphs of some of the experiments on dog 5 shown in figure 2. Although the changes are less striking when presented in this form, they are nevertheless discernible and present some additional points. In the first place these graphs emphasize that there was a far smaller absolute amount of potassium absorbed than of either sodium or chloride; in the second place the change on withdrawal of cortical hormone expressed itself in the diminished volume of fluid absorbed more markedly than in any change in concentrations; and finally, changes in osmotic pressure of the gut solution are best presented in this form. In general, in corroboration of Roepke and Visscher (16), the osmotic pressure dropped steeply in the presence of active absorption in the control experiments, but adrenal insufficiency uniformly delayed or abolished this fall, while cortin administration brought restoration approximately to the normal slope. Sulfate curves were omitted from the graphs of figure 2, but uniformly this ion underwent concentration in the control experiments, and fell in insufficiency.

In the condition of shock encountered in attempting acute experiments on adrenalectomized dogs, an exaggeration of the above-mentioned changes was encountered (fig. 3). In contrast to the control experiments, whether magnesium sulfate was present or not, the concentration of each constituent approached that in the plasma and the volume increased greatly, indicating that fluid was being poured into the lumen by the gut wall. The blood pressure fell to critical levels in the first few minutes (30 to 40 mm. of mercury) in the adrenalectomized dog, and sufficient hemoconcentration occurred during the procedures to raise the relative cell volume from 33 per cent to 67 per cent in 3 hours in spite of normal blood chemical findings. In control animals, on the other hand, subjection to the same manipulations resulted in no appreciable change in pressure or cell volume for over 4 hours.

Discussion. Whether our results in the acute experiment after adrenalectomy are due to adrenal cortical insufficiency is not entirely clear. The delay in fluid absorption and the rise in sodium level resemble greatly the changes seen in chronic loop dogs. Nevertheless, the hemoconcentration, the fall in blood pressure, and the approach of all ionic concentrations of the loop fluid to the plasma levels indicate that widespread changes had occurred. Certainly a falling blood pressure might introduce secondary effects which would confuse the direct adrenal insufficiency effect upon the process of intestinal absorption.

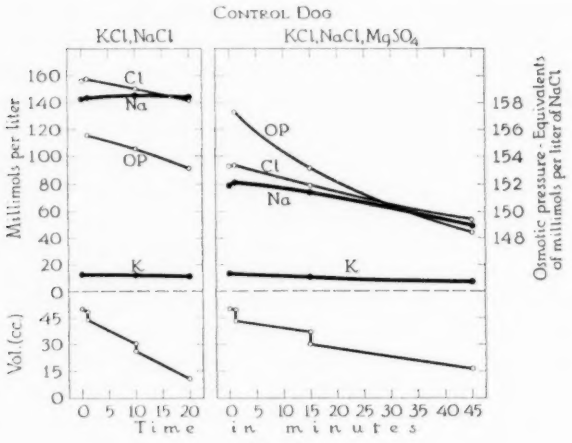

Fig. 3a

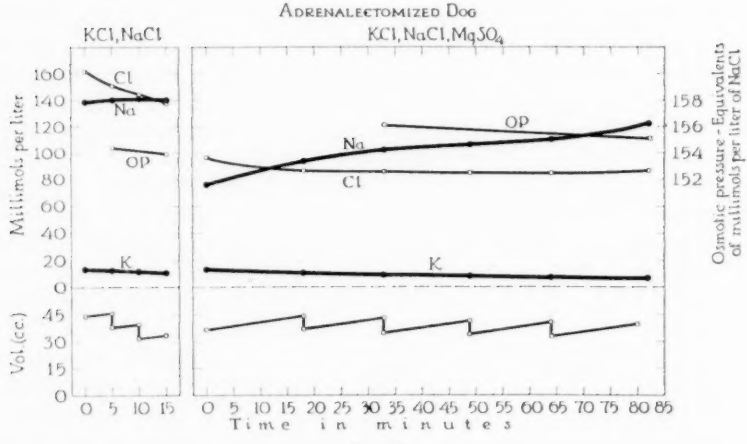

Fig. 3b

Fig. 3. Acute experiments in a control and an adrenalectomized dog maintained ten days without cortin showing the changes in volume, osmotic pressure, and concentrations of the various ions in solution in the gut fluid. Sample removals are indicated by vertical jogs in the volume curves. The blood chemical findings in the adrenalectomized dog were: At end of procedure: cell vol. 67 per cent, plasma chloride 100 mM., plasma sodium 126?mM., plasma potassium 4.1 mM., and B.U.N. 12.5 mgm. per cent; at start of procedure cell volume was 33 per cent. In the control dog: cell vol. 55 per cent, plasma chloride 101 mM., plasma sodium 145 mM. Plasma potassium 4.3 mM., and osmotic pressure the equivalent of 155.05 mM. NaCl.

In the absence of adrenal cortical hormone it is evident that, relative to sodium, the amount of potassium absorbed is increased. The changes found in absorption of potassium and sodium in the absence of adrenal cortical hormone are definite but small enough to lead to doubt that they could be solely responsible for the changes of adrenal insufficiency, though they may easily play a part. Harrison and Darrow (11) described changes in renal function, namely, decreased excretion of potassium and increased loss of sodium; these act in conjunction with the changes of ileal function we have described in adrenalectomized dogs to tend to produce accumulation of potassium and depletion of sodium in the body.

The changes observed might well be due, not to a specific interference with the handling of any one of these three ions, but rather to a general decrease in the ability to do osmotic work against concentration gradients. This is particularly evident in these experiments on observation of the direction of gradients: potassium toward the plasma in all cases, chloride toward the plasma and sodium equimolar with the plasma in the absence of magnesium sulfate, and both sodium and chloride toward the lumen in the presence of magnesium sulfate.

The experiments of Goldschmidt and Dayton (10) and studies from this laboratory (14) (9) on various segments of the intestine with regard to chloride absorption indicate inactivity high in the small intestine, and behavior similar to the ileum in the colon. It therefore seems not unwarranted to assume that in respect to the present study also, the ileum may be considered characteristic of the salt-absorbing area of the intestinal tract.

Harrison and Darrow used two days' treatment with cortical extract in their renal studies to restore normality. The data presented in figure 1 on cortin treatment suggest a progressive improvement in function without complete return to normal in forty-eight hours. This suggests that perhaps the time allowed was inadequate, and that these workers, as well as we, would have been able to duplicate their normal controls if longer periods of cortin treatment had been allowed.

Ingraham and Visscher (12) have pointed out that the impoverishment of uni-univalent salts in the presence of polyvalent ion salts is a phenomenon not limited to the ileum, but is found more generally. The greater impairment in re-absorption of sodium than of potassium ion found by Harrison and Darrow in the tubule and the findings for the ileum presented in this paper represent an additional point of similarity between these two structures.

SUMMARY

1. In adrenalectomized dogs maintained on high-sodium, high-bicarbonate, low-potassium diets following withdrawal of adrenal cortical

hormone, there is a very marked decrease in the rate of absorption of sodium, potassium, and chloride from chronic loops of ileum. This trend was in every case reversed by cortical hormone administration.

2. After cortical hormone withdrawal on the high-sodium, high-bicarbonate, low-potassium diet, the rate of sodium absorption in general declined more than that of potassium. The most striking observation was that in this state there was often an actual reversal in direction of net movement of sodium, which was sometimes excreted into the gut in relatively large quantity, although potassium was still being absorbed.

3. The decrease in osmotic activity of gut fluid seen in normal animals during absorption is less rapid after withdrawal of adrenal cortical hormone and dietary maintenance. This finding is interpreted to mean that the passive as opposed to the active processes in absorption are relatively more important.

4. In spite of essentially normal blood chemical determinations and objectively excellent health, the behavior of the intestine in the adrenalectomized dog could not be maintained normal in the absence of adrenal cortical hormone. The adrenalectomized dog, therefore, remains distinctly abnormal if adrenal cortical hormone is withheld.

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MASCULINIZATION OF FEMALE RATS TREATED WITH TESTOSTERONE PROPIONATE

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Administration of male sex hormone to pregnant female rats produces various degrees of intersexuality in the female offspring (1, 2). If treatment with testosterone propionate[1] is started in normal female rats immediately after birth, certain male structures develop, especially prostate (3, 4). At the same time, inhibition of female structures may take place (5, 6, 7). It appears that male sex hormone enhances development of the derivatives of the Wolffian duct and of the mesonephros, whereas inhibition of female structures is restricted specifically to the lower vagina. In this connection it is interesting to recall that Green, Burrill, and Ivy, believe the lower vagina is derived not from the Muellerian duct but from the urogenital sinus.

In female newborn rats treated with testosterone propionate from the first day of life as previously reported (7), we observed a male structure hitherto not described.

Animals were sacrificed at various stages of treatment from three and a half months up to thirteen months of age. The total dosage of testosterone propionate varied from forty-five to one hundred and fifty milligrams. On dissecting the anterior abdominal wall, a small sacculus was found distal to the inguinal fold, measuring about 0.5 to 0.75 cm. in length. This sacculus is apparently formed by an extension of the fascial layer of the abdominal wall. On opening this sacculus, one to two well-defined fat lobules were found. Through the abdominal wall they connected by a strand of connective issue and fat with the more diffuse fat bordering the uterus. A group of muscle fibers, varying greatly in number, branches off from the abdominal wall and enters the sacculus (fig. 1).

This brief description shows that the scrotal structures: Cooper's fascia, tunica vaginalis communis, and musculus cremaster were present. No scrotum was developed, but the inguinal canal was patent and the sacculus communicated with the peritoneal cavity. The fat lobules were

[1] We are indebted to Dr. Max Gilbert and Dr. Erwin Schwenk of Schering Corporation, Bloomfield, N. J., for our supply of testosterone propionate (Oretone).

bean shaped and well-defined, and in this respect differed markedly from the more diffuse fat surrounding the uterus. Fat lobules of this sort are frequently present in the scrotum of normal male rats. No testes, epididymis, or deferential duct were macroscopically visible, but it was as if everything were ready to receive these male structures.

Fig. 1. Anterior abdominal wall; fat lobule in sacculus

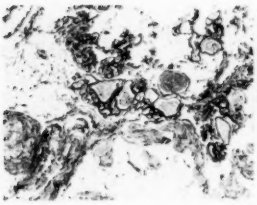

Fig. 2

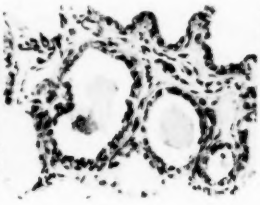

Fig. 3

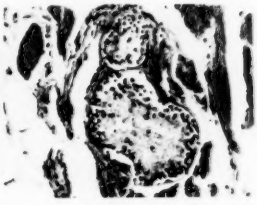

Fig. 4

Fig. 2. Rat 96 rh, 14 months of age, total dose 100 mgm. of testosterone propionate. Cross section of contents of the sacculus. Note numerous tubules. Zenker-Formol. Hematox.-Eosin 15×.

Fig. 3. The same preparation as figure 2, 165×.

Fig. 4. Rat 147 lflh. Fat lobule on the left, muscle layer (cremasteric muscle) on the right. Two tubules, lumen filled with desquamated cells and cell detritus. Zenker-Formol. Hematox.-Eosin. 125×.

The contents of the sacculus were examined histologically in eleven cases. In three of these tubuli were found. These tubuli were not located in the fat but in the connective tissue accompanying the cremasteric muscle. On cross sections they appear of various width. They were separated by thin layers of connective tissue. The epithelial lining was low columnar to cuboidal. No basal membrane was visible. Part of the epithelial cells was vacuolized. Some of the tubuli contained desquamated cells, cell

detritus, or more or less homogenous masses giving the impression of coagulated fluid (figs. 2, 3). In one of the three cases tubular development was much less advanced than in the other two and only two small tubuli were found (fig. 4). In none of the animals was there any trace of testicular tissue.

The question arises what structure these tubules represent. Their localization made us think of epididymis. According to the text-books on embryology, the epididymis is in part a derivative of the Wolffian duct and in part is derived from the mesonephros. We thought it probable that the male sex hormone administered from birth had stimulated development of a rudimentary epididymis which eventually descended into the sacculus representing scrotal layers.

Ivy in a personal communication points out that in the rat the epididymis is derived from the Wolffian duct only. As the portion of the Wolffian duct from which the epididymis develops is completely degenerated at birth, the tubules cannot in his opinion be homologues of an epididymis. He, too, considers the tubules to be of mesonephric origin but not a homologue of the epididymis.

Greene, Burrill and Ivy (1) have produced intersexed female rats by administering male sex hormone to the pregnant mother. In these animals, the derivatives of both the Wolffian duct and the Mullerian duct were developed including both oviduct and epididymis. The latter was located with the oviduct at the distal end of the uterine horn. The experiments reported in this paper indicate that in the rat, born as it is in a very immature stage, mesonephric tubules may be stimulated by male sex hormone treatment started immediately after birth. The inguinal canal opens into a sacculus representing scrotal layers. At this site in the sacculus, the mesonephric tubules were found. We cannot state whether they have developed there from aberrant mesonephric tubules accompanying the gubernaculum or whether they have developed higher up and descended into the sacculus imitating a descent of testes and epididymis. It should be noted that ovaries, oviducts, and uteri, were normally developed in all these animals.

We wish to draw attention to a further masculine feature observed in a large series of female rats treated with testosterone propionate from the first day of life. In male adult rats of our strain, the hair is considerably coarser than in females, and the color has a slightly yellowish tinge. In all our 32 females treated with testosterone propionate the hair turned coarse and yellowish—so much so that from these qualities one was always able to distinguish the treated animals from their untreated female litter mate controls. In another series of experiments, we administered large doses of estrogen to male rats over periods covering several months. However, none of these males acquired the female type of hair.

Relationship of male sex hormone to hair growth is well established in man inasmuch as beard and body hair is concerned. Apart from this, comparatively little is known of the influence of sex hormones on hair growth. Tandler and Gross (8) observed very dense growth of the head hair in castrates (Skops) and quote a similar observation by Pelikan (1876). Greenhill and Freed (9) observed hirsutism in two women after treatment with therapeutic doses of testosterone propionate (450 and 300 mgm. respectively). Olivet (10) claims that female type of hair growth is independent of hormonal influence, whereas the male type of hair growth is dependent on pituitary action.

From our observations we cannot say whether the male sex hormone influences the type of hair growth directly or whether this influence is exerted by way of the pituitary. But we can add weight to Olivet's hypothesis inasmuch as male sex hormone produces heterosexual (i.e., male) type of hair in the female rat, whereas female sex hormone fails to influence the male type of hair in the male.

SUMMARY

Two masculine features in female rats treated with testosterone propionate are described. A. Development of scrotal layers containing tubuli which apparently represent rudimentary mesonephric tubules. B. Coarse masculine hair.

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AN ANALYSIS OF THE EFFECT OF CARBON MONOXIDE ON THE RESPIRATION OF FROG SKELETAL MUSCLE

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Fenn and Cobb (1932a) were the first to study carefully the effects of carbon monoxide on the respiration of muscle. They found that instead of inhibition an apparent stimulation of the oxygen consumption followed treatment with various CO-O_2 mixtures. The stimulation occurred at all concentrations of CO studied as long as oxygen was present also, but was maximal in mixtures consisting of 80 per cent CO and 20 per cent O_2, in which case the increase in metabolic rate was over 100 per cent of the basal level. Subsequently Fenn and Cobb (1932b) showed by direct analysis of the gases used that the stimulation resulted from oxidation of the CO to CO_2. This property is apparently limited to skeletal and cardiac muscle and contrasts markedly, of course, with the usual effects of CO on tissue respiration.

The stimulation of the oxygen consumption of muscle by carbon monoxide was confirmed by Schmitt and Scott (1934). More detailed studies of the effect were made by Carleton (1935) and Carleton and Fenn (1938). In addition Hursh (1936) found that stimulated muscle showed a *diminished* excess oxygen consumption and a diminished recovery heat production in 79 per cent CO-21 per cent O_2 compared with muscle stimulated in air. Since there was no accumulation of lactic acid in the muscle recovering in CO, Hursh concluded tentatively that the effect should be described as an increase in the efficiency of the recovery processes in CO.

In connection with a general study of the oxygen transfer mechanisms in frog muscle (Stannard, 1939a, b) further analysis of these effects of CO seemed important. Since frog muscle is known to contain cytochrome and cytochrome-oxidase (Keilin, 1925) and its respiration is almost completely cyanide-labile (Stannard, 1939a) a primary problem was to determine whether or not this response to CO represented some unique variation in the functioning of the Warburg-Keilin system or an independent enzyme system. It is believed that the experiments reported in this paper provide

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proof that the oxidation of CO is brought about by processes separable from, but similar to, the cytochrome-cytochrome oxidase system and furthermore that the cytochrome oxidase is really inhibited by CO in frog muscle, just as in test tube preparations or yeast in spite of the increased gas consumption in the presence of CO, seen in muscle.

METHODS. The oxygen consumption was generally measured in differential volumeters in principle like those described by Fenn (1927) but fitted with two side-arms, one for alkali, the other arranged for tipping reagents into the main chamber easily, and a side-tube with stopcock venting to air to facilitate the passage of gas mixtures.

When it was desired to analyze the gaseous contents of the respirometer bottle after an experiment a special respirometer was employed similar to that described by Fenn and Cobb (1932b). This enabled measurement of the total gas used over a long period of time without opening the apparatus to the air since the index drop could be reset simply by introducing mercury from a reservoir to diminish the volume of the gas space. The apparatus was arranged so that mercury never came in contact with the muscle during an experiment. After a sufficiently long experimental period to insure an easily measurable change in the composition of the contained gases, the whole apparatus could be filled with mercury and the gas displaced into a mercury-filled sampling tube for analysis. The final analyses were always made in duplicate and often in triplicate. This technique requires close attention to detail for, at best, the difference in gas compositions before and after an experiment is less than 3 per cent of an atmosphere (average about 1.3 per cent). However, the largest variation tolerated between analyses was 0.30 per cent (one instance only) and the average variation was 0.08 per cent oxygen. On the average there is thus a 6 per cent inaccuracy due to errors in the gas analyses when the special technique is used. The inaccuracy in operation of the volumeter is of about the same order. Thus, if all errors summed, they might amount to as much as 12 per cent, but on the average considerably less would be expected. It will be noted that the differences quoted are of sufficiently greater magnitude to establish their reality.

The carbon monoxide was prepared in the usual manner from formic acid and passed through strong alkali before collection in siphon bottles over dilute alkali. The usual thin hind-limb muscles of summer and winter frogs were dissected the evening before an experiment and kept in oxygenated Ringer's solution at 5°C. overnight. The muscles were thus in a truly resting state at the start of each experiment (cf. Stannard, 1939a). This is important since, as will be shown, the general level of metabolism influences the results. Four pairs of matched muscles were compared with each other in each experiment. In addition the gas mixtures were often interchanged and other measures taken to completely eliminate any

possibility that the results could be due to chance variations in the behavior of individual muscles. The temperature was 22° or 23°C. ± 0.01°C, For other details including the composition of the Ringer's solution. preparation of reagents, see Stannard (1938, 1939a).

RESULTS. 1. *Is the stimulation really due to oxidation of the CO?* In their original study of the effect of CO-O_2 mixtures on muscle Fenn and Cobb excluded most of the more obvious possible explanations for the stimulation by CO. Then it was shown experimentally that the stimulation was due to oxidation of the CO. However, since the burning of CO by animal tissues seems rather improbable from a teleological standpoint the original observations have been received with some incredulity. Therefore, a number of control experiments were carried out which should be reported since each upheld the validity of the methods used.

a. The absorption of CO as formate by the KOH may be a source of error in manometric experiments with CO. Controls were therefore arranged identical with the standard experiment but without tissue. In three such experiments the movement of the drop indicated a diminution in volume at the rate of 0.36 mm^3/hr. (maximum 0.51 mm^3/hr.). This figure includes all errors such as diffusion of gas through stopcocks, temperature inequalities, etc., in addition to the combination of CO with the alkali. The average excess respiration with muscle present (200 mgm.) is about 10 mm^3/hr. or many times the drift found above without tissue.

b. The difference in solubility of CO and nitrogen can account for a total of only about 6 mm^3 of gas (cf. Carleton, 1935). Thus at 22°C. the solubility of CO is 0.0224 volumes per volume of water per atmosphere pressure while that of nitrogen is 0.015. With 1 ml. of fluid and 80 per cent CO or nitrogen, as the case may be, a net exchange of (22–15) 0.8 = 5.6 mm^3 would be expected. The same ratios of the solubilities would be expected in Ringer's solution as in water. Since at least a liter of gas is run through the respirometers this exchange should be complete before the readings are begun.

c. Haldane and Priestley (1935, p. 238) state that Fenn and Cobb's experiments are invalid since they give no evidence that they allowed for the fact that CO is much more soluble than nitrogen, or for "combination of CO with cytochrome." These objections are not valid. The error due to the difference in solubility of CO and nitrogen in the fluid in the respirometer bottle has been discussed above. Increased solution of CO as compared with nitrogen in the very alkaline solutions in the Haldane gas analyzer, especially by the formation of formate, might be a serious error. However, as stated earlier, the analyses were always made in duplicate or triplicate and agreed well with one another. Also, the comparison is between mixtures of only slightly different CO contents. Actually when analyses of N_2-O_2 mixtures immediately preceded analyses of CO-O_2 mixtures the first analysis of the latter was often higher than subsequent ones. However, any further analyses of this mixture agreed satisfactorily and the first figure could be discarded.

With regard to the second point raised by Haldane and Priestley, if we assume that even the entire iron content of frog muscle (0.83 mgm. Fe/100 gram muscle, Fenn, 1935, p. 479) combines with CO in the ratio of one CO to one Fe only 3.2 mm^3 of CO per gram of muscle would disappear. This amount could account for less than four minutes of the increased gas consumption in CO.

d. A further proof of the validity of the method is found in the fact that tissues which show no stimulation in CO show no large difference between the total gas disappearance and the oxygen utilized (cf. Fenn and Cobb, 1932b, table 2). In my experiments yeast in glucose-phosphate was tried and showed less than 5 per cent difference between the total gas consumed and the oxygen disappearing. When the entire procedure was carried out on muscles in air the agreement was within 4 per cent (one experiment).

Thus there remains little reason to doubt that the stimulation of muscle respiration represents oxidation of the CO to CO_2, and it will be referred to as such subsequently.

In table 1 five experiments on resting muscle at pH 7.38 are presented along with data taken from Carleton's dissertation (1935) at pH 4.46 and 6.01. For calculation the following expression was used:

$$O_2 \text{ used} = V(x - z) + z\Delta V$$

$$CO \text{ used} = \Delta V - [V(x - z) + z\Delta V]$$

where V = initial volume of gas, x = initial per cent O_2, z = final per cent O_2, ΔV = diminution in volume during the experimental period.

All volumes are of moist gas at the temperature and pressure of the experiment. This was considered a more accurate equation than the one employed by Fenn and Cobb (1932b) and Carleton's data were recalculated on this basis. The "calculated excess" equals 1.5 times the amount of CO disappearing to account for the half mol of oxygen needed for each mol of CO oxidized. This calculated excess agrees well (pH 7.38) with the average stimulation (50 mm^3/g./hr.) measured in ordinary respirometer experiments. Fenn and Cobb obtained an average stimulation of 64 mm^3/g./hr. in ordinary respirometers. Calculation of the excess expected from their experiments with gas analyses (table 2, 1932b) indicates an average excess of 40 mm^3/g./hr.

2. *The CO excess after the addition of substances which increase the rate of respiration.* The oxidation of CO seemed at first to function best in resting muscle. Thus in the earlier work of Fenn and Cobb a decrease in the CO effect appeared at high concentrations of KCl, in spontaneously non-irritable muscle, and in muscle mash. In my earlier experience freshly dissected muscle showed only small increases in respiration when put into CO-O_2 mixtures. In each of these conditions the basal rate of oxygen

consumption is already high. Hence it seemed possible that the unused cytochrome oxidase in truly resting muscle might accomplish a union of CO with "activated oxygen" when the system is unsaturated with the normal substrate but not when the system is saturated. Such a decrease in CO burning at high rates of oxygen consumption might readily account for the results of Hursh (1936, especially p. 632).

TABLE 1

*Amount of CO burned by resting muscle in 80 per cent CO-20 per cent O_2**

pH	CONTROL	TIME	x	z	ΔV	OXYGEN USED	CO USED	CO / O_2	CALC. EXCESS
	$mm^3/g./hr.$	*minutes*	*per cent*	*per cent*	*ml.*	*ml.*	*ml.*	*per cent*	$mm^3/g./hr.$
7.38	36	425	20.40	19.70	0.755	0.381	0.374	98	95
	65†	530	16.25	15.50	0.616	0.342	0.274	80	42
	79†	400	16.56	15.53	0.580	0.419	0.161	38	33
	27	422	19.41	18.96	0.643	0.263	0.410	156	57
		420	18.08	16.83	0.972	0.558	0.414	74	48
Average								89	55
6.01‡	15.5	540	18.41	18.05	0.395	0.176	0.219	124	
	40	540	18.01	17.20	0.588	0.335	0.253	76	
	20	450	17.06	16.66	0.307	0.168	0.139	83	
	31	600	18.31	17.44	0.536	0.346	0.190	55	
Average								85	
4.46‡	25	690	17.50	17.07	0.540	0.180	0.360	200	
	35	600	26.60	25.82	0.478	0.335	0.143	43	
	39	600	19.72	18.78	0.633	0.399	0.234	59	
Average								100	

* The values for V (equations 1 and 2) are 33.194, 32.91, 32.93, 31.32, and 31.64 ml. in the uppermost group of experiments. Similarly the weights of muscle used were 0.838, 1.120, 1.106, 1.516, 1.860 grams respectively.

† Rate in CO/O_2 mixture by parallel experiment, others in air.

‡ Calculated from data obtained by Carleton (1935) and hitherto unpublished. The rate of CO utilization could not be calculated because the weight of tissue used was not available.

Later Carleton and Fenn (1938) found the excess metabolism due to CO was little if any modified by the large increase in metabolism seen in KCl at concentrations above 0.02 M, except possibly at the peak of the KCl effect when there was a slight decrease in the CO excess. In addition both the normal and CO respiration were similarly affected by changes in pH.

A more complete study of the relation between CO oxidation and the basal respiration has been made here to test the hypothesis outlined above.

Table 2 presents a summary of experiments in which the rates of respiration were measured before and after addition of the substance used to raise the metabolism and again after the addition of CO. Controls, not shown in

TABLE 2

The CO excess in 80 per cent CO/20 per cent O_2 mixtures after addition of substances which increase the rate of oxygen consumption

SUBSTANCE	CONCENTRATION	NUMBER OF EXPERIMENTS	BEFORE ADDITION	AFTER ADDITION	WITH CO	EXCESS
			$\overline{mm^3/g./hr.}$	$\overline{mm^3/g./hr.}$	$\overline{mm^3/g./hr.}$	$\overline{mm^3/g./hr.}$
Lactate	0.2%; 2%	4	41.5	76	109	+33
Pyocyanin	0.005%	1	41	92	119	+27
KCl*	0.0015–0.116M*	21*	40*	123*	166*	+43*
o-Chlorophenol indophenol‡	0.0003–0.0017%‡	4‡	52‡	82‡	129‡	+47‡
Dextrose	4.5%	6†	40	77	75	−2
Dextrose + NaCl	4.5%	4	40	39	63	+24
Methylene blue	0.001M	3	29	154	153	−1
p-Phenylene-diamine	0.001M	4	22	66	65	−1
Nadi reagent	0.001M	2	44	78	78	0
Caffeine	0.04%	5	37	139	143	+4

* Data of Carleton and Fenn (1938).

† One experiment with isotonic sucrose, but no difference in results.

‡ Data of Carleton (1935). The results were qualitatively similar at all concentrations of the dye used.

TABLE 3

Apparent effect of CO on caffeinized muscle (80 per cent CO/20 per cent O_2)

O_2 CONTENT	WITHOUT CO	WITH CO	EXCESS	DIFFERENCE
per cent	$\overline{mm^3/g./hr.}$	$\overline{mm^3/g./hr.}$	$\overline{mm^3/g./hr.}$	*per cent*
20	107	110	+3	+3
20	143	152	+9	+6
20	154	165	+11	+7
22	154	139	−15	−10
24	138	151	+13	+9
Average	139	143	+4	+3

the table, included measurements of the increment due to CO in untreated muscles. Table 3 shows the individual experiments with caffeinized muscle for use in a later connection and to indicate the range of variation in a given series (of which it is typical). Superficially it appears that the excess metabolism in CO persists in spite of increases in the basal rate of

respiration by lactate, pyocyanin, KCl, and o-chlorophenol indophenol, but not in the other cases.[2] At first glance it might seem that the latter data indicate only a "ceiling effect," *viz.*, the rate of respiration could be increased no more at the prevailing O_2 tension, etc. In fact in some experiments with lactate this explanation appeared to be valid. Thus at 2.3 per cent lactate (not shown in the table) the basal rate of 136 mm^3/g./hr. increased to only 139 in 80 per cent CO-20 per cent O_2 mixture. At lower rates of respiration with 0.2 per cent lactate there was a definite increase after addition of CO (table 2). However, this cannot be a general explanation of the data in table 2, since, for example, with isotonic dextrose the basal rates are actually lower than with KCl, yet no increase due to CO was apparent. Further evidence that the respiration had not reached a peak or "ceiling" before the addition of CO is seen in the following facts: 1, the rate in dextrose was doubled by the addition of methylene blue; 2, raising the O_2 tension did not increase the rate in dextrose alone. It should be added that the muscles were of approximately the same thickness in all experiments.

Thus, the data of table 2 indicate that the effect of CO is not directly conditioned by the prevailing rate of oxygen consumption, nor is there any evidence that substances which augment the activity of the cytochrome-cytochrome oxidase system interfere uniformly with the burning of CO. Thus the increased respiration caused by caffeine, Nadi reagent, p-phenylene diamine, KCl, and lactate are all inhibited completely by sodium azide and cyanide (Stannard, 1939a, b). Yet, the KCl and lactate do not prevent stimulation by CO while the remainder appear to accomplish this. The exact reason for the differences in this respect between the upper and lower groups in table 2 is not entirely clear and awaits future study.

However, before such a study could be undertaken other factors made it obvious that the differences were more apparent than real. In fact, CO burning probably occurred in all cases but was masked in some cases by a corresponding inhibition of the basal O_2 consumption. Experiments suggesting this interpretation will be considered in the following sections.

3. *The effect of high CO tensions and of light.* In none of the experiments gathered in table 2 is there any indication that CO may *inhibit* respiration even at the highest rates where the Warburg-Keilin system is presumably fully active. If the cytochrome-cytochrome oxidase system is functional in frog muscle and comparable in properties to that well known in many

[2] It will be noted that the addition of sodium chloride to muscles in isotonic dextrose brings the rate of oxygen consumption back to the resting level. This effect is well known. The striking fact here is that with the return to resting levels the increment due to CO reappears. The final concentration of NaCl was 0.05 *per cent*.

tissues and microörganisms why should there be no evidence of inhibition by CO at high rates of oxygen consumption? An obvious possibility, and one entertained by Fenn and Cobb and by Schmitt and Scott, is that the burning of CO is superimposed upon inhibition of the normal respiration. The measured rate of respiration would then be the resultant of inhibition and stimulation.

The first test of theory was made by raising the CO tension of the gas mixtures in those cases where 80 per cent CO-20 per cent O_2 mixtures had no apparent effect. It was thought that this procedure might throw into relief any inhibition due to CO. But, as shown in table 4, raising the CO content to 95 per cent led to no essential change in results with Nadi reagent, dextrose, or caffeine when compared with nitrogen-oxygen mix-

TABLE 4

*The effect of higher CO tensions on muscle in Nadi reagent, dextrose or caffeine**

SUBSTANCE ADDED	O_2 CONTENT OF MIXTURE	RESPIRATION		CHANGE IN CO	
		N_2-O_2	CO-O_2		
	per cent	*mm³/g./hr.*	*mm³/g./hr.*	*mm³/g./hr.*	*per cent*
Nadi reagent	20	91	80	−11	−12
	10	82	86	+4	+5
	5	60	68	+8	+13
Dextrose	10	46	47	+1	+2
	5	43	57	+14	+33
Caffeine	5	72	80 (D)†	+8	+11
		73	87 (L)†	+14	+19

* Comparison was always between N_2-O_2 and CO-O_2 mixtures of same O_2 content.
† (D) = dark, (L) = light from arc lamp (see text).

tures of same oxygen content. If any greater inhibition of the cytochrome system did occur at these tensions, the burning of CO must have increased in proportion.

Some evidence of inhibition was obtained, however, by exposing caffeinized muscle in 95 per cent CO-5 per cent O_2 to light from a 7 ampere arc placed 11 cm. from the respirometer. Since light is known to release the cytochrome oxidase (or at least pheohemin) from inhibition by CO the light acceleration (cf. table 4) suggests that some inhibition by CO was occurring. Increases of this order were found in other cases even with 20 per cent oxygen. Ordinary daylight had no effect. However, the necessary light intensity for pronounced effects on tissues was not available.

This experiment is consistent with and confirms Schmitt and Scott who found a greater excess due to CO at high CO low O_2 tension in strong light (30–60 ampere arc) than in the dark. In other tissues which do not appear

to burn CO in the dark or ordinary daylight there was an augmentation *above* the rate in air in the presence of CO and bright light. Although it may become necessary to assume that the catalyst for CO burning is itself accelerated by light in certain instances most of these effects can be interpreted as a simple release of inhibited respiration so that the resultant of inhibition and stimulation is increased in magnitude.

4. *CO inhibits the production of indophenol blue from Nadi reagent.* More definite evidence of an inhibitory effect of CO was found in further experiments with the Nadi reagent. It was noted above (tables 2 and 4) that CO does not appear to exert any marked effect on the oxygen consumption even in the presence of the Nadi reagent. This was puzzling, especially at high CO tensions since the oxidation of the Nadi reagent is rather specifically mediated by cytochrome and cytochrome-oxidase in conjunction (Stotz, Sidwell and Hogness, 1938). However proof that the production of indophenol blue from Nadi reagent was actually considerably retarded by CO in spite of the evidence from oxygen consumption measurements was found in test-tube experiments with cut-up gastrocnemius muscles. The entire procedure of the respirometer experiments was repeated except that the production of color was observed and compared roughly with the oxygen consumption measurements on muscles from the same frog in the same gas mixture. Thus in one experiment the rates of oxygen consumption with 0.001M Nadi reagent were 88 mm^3/g./hr. with both 90 per cent CO and 90 per cent N_2 in oxygen. After interchanging the gas mixtures the muscles in N_2-O_2 consumed oxygen at the rate of 73 mm^3/g./hr. and those in CO-O_2 at 83 mm^3/g./hr. In the test tubes equilibrated with the same gas mixtures and kept in the dark a large amount of indophenol blue had been formed after 35 minutes in the N_2-O_2 mixture while only traces had appeared in the CO tube. Light from the arc caused a rapid irreversible color production in this tube so that in five minutes the two tubes were identical in color. In another experiment with 5 per cent oxygen in the mixtures the measured rates of respiration were 60 and 68 mm^3/g./hr. in N_2 and CO mixtures respectively. Both tissue and solution were dark blue in the nitrogen-oxygen tube while the CO-oxygen tube was but faintly tinted after one and a half hours in the dark. Bright light equalized the colors in about ten minutes. Similar though not so striking results were obtained with 20 per cent oxygen in the mixtures.

The most logical interpretation of these facts is that CO inhibits that fraction of the oxygen transfer mediated by the cytochrome-cytochrome oxidase system (exclusive of the autoxidizable cytochrome-B) presumably as a function of the CO tension, but is burned in equivalent amounts up to a limit set by one or more factors. The resultant often represents a rate of respiration nearly identical with that in the absence of CO.

5. *Gas analyses.* Perhaps the best method of demonstrating that CO

is burned even when no stimulation of respiration by CO is apparent is by comparison of the total gas disappearing with the oxygen consumed when rapidly respiring muscles are exposed to CO-O_2 mixtures and nitrogen-oxygen mixtures. For this purpose the special apparatus and technique described above (cf. methods and section one) were employed. It would be desirable to use Nadi reagent in these experiments for comparison with the observations on color production. However caffeinized muscle is more convenient because the high rate of oxygen consumption is better maintained. This is important because an experimental period of five or six hours is required to insure an adequate change in the composition of

TABLE 5

The amount of CO burned by caffeinized muscle in 80 per cent CO-20 per cent O_2 (0.04 per cent caffeine) *

CONTROL	x	z	ΔV	OXYGEN USED	CO USED	$\frac{CO}{O_2}$	CALC. EXCESS
$mm^3/g./hr.$	*per cent*	*per cent*	*ml.*	*ml.*	*ml.*	*per cent*	$mm^3/g./hr.$
148	23.13	21.14	0.975	0.814	0.161	20	42
143†	26.16	24.70	0.925	0.690	0.235	34	73
154	22.04	20.40	0.790	0.686	0.104	15	44
138	23.97	22.71	0.666	0.554	0.112	20	42
Average						22	51.5
202‡	21.01‡	19.09‡	0.803‡	0.770‡	0.033‡	4.1‡	9‡

* The values for V (see equations 1 and 2) were 30.497, 31.762, 31.876, 32.025, and 32.121 ml. reading from above downwards. Similarly the weights of muscle were 1.585, 1.070, 0.956, 0.807 and 0.711 grams respectively. The control values (first column) refer to parallel determinations in ordinary respirometers.

† In CO/O_2.

‡ Control experiment with 80 per cent N_2-20 per cent O_2. The CO used and CO/O_2 columns are calculated as if CO were present.

the gases for analysis. Caffeinized muscle should be strictly comparable with muscle in Nadi reagent, however, since (tables 2 and 3) CO has little apparent effect on the oxygen consumption in either case in an ordinary respirometer experiment.

The results are collected in table 5 using equations (1) and (2) for calculation. Here it is seen that the total change in volume (Δ V) due to the metabolism of caffeinized muscle in 80 per cent CO-20 per cent oxygen cannot be accounted for entirely by the oxygen utilization. The difference is experimentally significant, although it is a lower percentage of the total since the basal rate of oxygen consumption is much higher than in the experiments with resting muscle (see table 1). In fact CO disappears at nearly the same *absolute* rate as with resting muscle. As shown in the

last column the "calculated excess" (1.5 times the CO disappearing) expressed as a rate agrees very well with the same figure found for resting muscle (55 mm^3/g./hr.). An experiment with an N_2-O_2 mixture (bottom of table 5) shows that in this case the total gas disappearing is accounted for as oxygen within 4.1 per cent.

Reference to table 3 shows an average *rise* in respiration of only 6 mm^3/g./hr. when caffeinized muscle is treated with this concentration of CO. Therefore the difference 51.5 − 6.0 = 45.5 mm^3/g./hr. must represent inhibition of the normal respiration of caffeinized muscle by the CO. Since the average rate of oxygen consumption of caffeinized muscle in air (data of tables 3 and 5) is 142 mm^3/g./hr. the percentage inhibition by CO is $\frac{45.5}{142} \times 100 = 32$ per cent, a not unlikely figure for tissues at 0.8 atmosphere tension of CO.

Analyses of the total gas versus oxygen consumption at higher CO tensions have not been made as yet. It is expected that no essential change in the results would ensue with caffeinized muscle for below (and often at) 20 per cent O_2 the overall rate of respiration in N_2-O_2 mixtures is limited by oxygen tension. Likewise the rate of CO oxidation diminishes as the oxygen tension is decreased below 20 per cent (Fenn and Cobb, 1932a, fig. 2). Thus, both processes might be expected to bear the same relation to each other at lower oxygen tensions although experimental proof is not yet available.

The experiments presented in this section and in section 4 are taken as evidence that the inhibitory effect of CO on oxidations mediated by the cytochrome-cytochrome oxidase system actually occurs in frog muscle but is masked by oxidation of CO, sometimes at rates such that the overall rate of respiration remains unchanged. To what extent the data quoted may be applied to muscles in dextrose, methylene blue, or other stimulants of oxygen consumption awaits future study. It does appear that CO is oxidized about as readily in muscle treated with KCl, pyocyanin, o-chlorophenol indophenol, Nadi reagent, lactate, or caffeine as by resting muscle. Considering the wide variety of these reagents tentative extension of the interpretation to dextrose and methylene blue does not seem entirely unjustified.

6. *The effect of chemical inhibitors.* Fenn and Cobb (1932a) found that the oxidation of CO by muscle was sensitive to cyanide (M/50 KCN reduced both the normal and CO excess respirations by about 90 per cent). Sodium brom-acetate (0.03 per cent) had no effect on either the normal respiration or CO oxidation. Hursh (1936) found that the effect of CO persisted undiminished in IAA-muscle.

These observations have been confirmed and extended to new inhibitors in the present work. The oxidation of CO can be almost completely

prevented by 10^{-3} M KCN. Thus in two experiments at 10^{-2} M KCN (balanced KCN-KOH mixtures in the side arms and KCN on the tissue) the CO excess was inhibited 99.5 and 101 per cent respectively. At 10^{-3} M the inhibitions were 90 and 88 per cent. The resting respiration is diminished, but to a lesser extent at these concentrations.

A study of the effects of iodoacetate and iodoacetamide showed that concentrations which have no effect on the resting respiration but com-

TABLE 6

The effect of iodoacetamide (CH_2ICONH_2) on CO oxidation in resting muscle (80 per cent CO-20 per cent O_2)

FINAL CONCN.	OXYGEN CONSUMPTION				CHANGE IN CO
	Resting In O_2-N_2	After iodoacetamide			
		In O_2-N_2	In O_2-CO	Excess in CO	
mM/liter	$\overline{mm^3}$/g./hr.	$\overline{mm^3}$/g./hr.	$\overline{mm^3}$/g./hr.	$\overline{mm^3}$/g./hr.	*per cent*
0.0091	29	29	57	+28	+96
0.017	29	30	66	+36	+120
0.018	38	39	71	+12	+31
0.033	23	31	65	+34	+110
0.17	24	21	76	+55	+260
0.18	32	30	75	+45	+150
0.18	26	38	52	+14	+37
0.99	30	25	24	−1	−4
1.0	22	19	30	+11	+58
1.8	33	16	7	−9*	−56*
5.0	22	10	8	−2*	−20*
	31	19	16	−3*	−16*
10	22	9	7	−2*	−22*

* These do not indicate *inhibitions* by CO (see text) because the CO-O_2 values were obtained subsequent to the N_2-O_2 values.

pletely inhibit the anaerobic glycolysis (cf. Stannard, 1938) do not interfere with the oxidation of CO by resting muscle. However, observations on iodoacetamide (IAAm), gathered together in table 6, showed that when the concentration was raised until the resting respiration was definitely decreased, the CO oxidation was effectively prevented. In all of the data shown in table 6 the CO excess seems to be slightly less than normal, but the variation was within the range of random errors. The CO itself seems to cause inhibition at the higher concentrations of iodoacetamide but this represents only the decline in rate with time which is characteristic

of higher concentrations of these substances and occurs to an equal extent in air. The measurements in CO were made by changing to CO-O_2 after the effect of IAAm in N_2-O_2 had been measured for about an hour. The decline in rate in air after iodoacetamide was determined by control experiments (cf. also Stannard, 1938).

In a recent publication (Stannard, 1939a) it was shown that sodium azide (NaN_3) is a more specific inhibitor than cyanide for the cytochrome oxidase in frog muscle. The resting respiration is only slightly affected by this reagent while the extra oxygen consumption due to stimulation is markedly affected. This does not mean that sodium azide can be consid-

TABLE 7

The effect of sodium azide on the excess metabolism in 80 per cent CO-20 per cent O_2

NaN_3 CONCN.	NUMBER EXPERIMENTS	OXYGEN CONSUMPTION						INHIBITION OF CO EXCESS‡
		Without azide			With azide			
		Resting	+CO	Excess due to CO	Resting	+CO	Excess* due to CO	
mM/liter		$mm^3/g./hr.$	$mm^3/g./hr.$	$mm^3/g./hr.$	$mm^3/g./hr.$	$mm^3/g./hr.$	$mm^3/g./hr.$	*per cent*
210	1	43	72	29	49	43	−6	108
10	1	30			28	37	+9	
7.2	1	22	64	42	30	23	−7	111
4.3–3.2	6	30	77	47	40	46	+6	92
2.4–1.9	7	29	66	37	49	54	+5	92
0.43	1	30	74	44	52†	67	+15	71
0.043–0.040	2	32	87	55	34	66	+32	42

* Calculated as above rate with azide alone.

† The experimental value with azide alone was excessively high in this experiment. Hence an average figure taken from a long series of experiments (Stannard, 1939a) was substituted in calculation.

‡ Calculated as $100 - \left(\frac{\text{Excess due to CO in } NaN_3}{\text{Excess due to CO without } NaN_3} \times 100\right)$.

ered truly specific for cytochrome oxidase in muscle and indeed there was an eventual effect even on the resting respiration, but its possible effect on the CO excess was, nevertheless, considered of more than ordinary interest. The data are gathered in table 7. In these experiments the stimulation by CO alone was determined separately, thus enabling a direct comparison of the effect of CO on the same muscles with or without azide. It will be noted that the lack of inhibitory effect of azide on the resting respiration is confirmed in these experiments. (The rise in resting respiration after azide in the middle concentration ranges is due to the slight contracture caused by this reagent.) On the other hand, the increment in respiratory rate caused by oxidation of CO is almost completely inhibited above 0.001 M azide. The diminution is rapid and occurs long

before the resting respiration is at all affected. Therefore the catalyst for CO burning cannot be identical with that responsible for the resting respiration.

Because of a possible connection between the CO burning and catalase activity the effect of hydroxylamine was tried (see discussion). Two sets of data are shown in table 8, one set at pH 7.3 (carefully maintained),

TABLE 8

The effect of hydroxylamine on the CO excess (80 per cent CO/20 per cent O_2)

EXPERIMENT	NH_2OH CONCENTRATION	OXYGEN CONSUMPTION IN						INHIBITION OF CO EXCESS*
		Without NH_2OH			With NH_2OH			
		Resting	+CO	Excess due to CO	Resting	+CO	Excess due to CO	
	mM/liter	*mm^3/g./hr.*	*mm^3/g./hr.*	*mm^3/g./hr.*	*mm^3/g./hr.*	*mm^3/g./hr.*	*mm^3/g./hr.*	*per cent*
1	2.1	30	81	51	29	40	11	78
2	2.1	54†	78	24†	42	32	−10	142†
3	4.3	21	91	70	37	43	6	91
4	2.1	33	79	46	37	42	5	89
	21.0	36	92	56	41	32	−9	116
5	2.6	22	73	51	36	43	7	86
6‡	0.042	39	79	40	44	96	52	−30
	0.42	45	79	34	44	86	42	−24
7‡	2.1	29	75	46	32	78	46	0
	21.0	29	75	46	33	45	12	74
8‡	0.036	36	85	49	37	75	38	22
	0.36	38	95	57	43	77	34	40
9	3.7	30	61	31	36	52	16	48

The data in the upper group were taken at pH 6.3, those in the lower group at pH 7.3. The concentration of NH_2OH refers to the final concentration assuming 80 per cent of the muscle wet weight is water. The effect of CO was compared on paired muscles with or without NH_2OH.

* Calculated as $100 - \left(\frac{\text{excess due to CO in } NH_2OH}{\text{excess due to CO without } NH_2OH}\right) \times 100$.

† Abnormal figures. The resting respiration was abnormally high. If an average figure be substituted the inhibition becomes 96 per cent.

‡ Average of two respirometers for each figure in these experiments.

the other at pH 6.3 the final H+ ion concentration reached when unneutralized stock $NH_2OH \cdot HCl$ was diluted to the required concentration with Ringer-phosphate. The effects resemble those of azide.[3] Low pH seems to favor somewhat inhibition of the CO excess by hydroxylamine. No data were obtained with azide at low pH. However the effect of pH

[3] The experiments with azide were all carried out at pH 7.38.

is not due to the acidity alone but to a specific inhibitory action of the reagent, since Carleton and Fenn (1938) found no specific effect of H+ ion concentration in this range on the CO burning. It is noteworthy that hydroxylamine, like NaN_3, does not decrease the resting respiration at concentrations which have a large effect on CO oxidation.

7. *Comparison of the effects of inhibitors on CO excess and caffeine excess.* These experiments on inhibitors lead to an apparent paradox. The effect of sodium azide has been attributed in earlier reports to its action on the cytochrome oxidase. It is found in this work to inhibit the CO excess. Yet the other evidence presented here indicates that the oxidation of CO is superimposed on inhibition of the cytochrome system! This confusion was eliminated by a comparison of the relative effects of a given concentration of the inhibitor on the CO excess and on the extra respiration due to increased activity of the cytochrome system (e.g., by means of caffeine).

In the experiments with azide there were indications that the oxidation of CO was more sensitive to this reagent than the extra respiration of caffeinized muscle. Thus 4×10^{-5} M azide has relatively little effect on the latter (cf. Stannard, 1939b, fig. 2), yet it inhibits the CO excess by 42 per cent. With hydroxylamine this difference in relative sensitivity was striking. Thus in experiment 5 shown in part in table 8 paired muscles were treated with caffeine and exposed to the same concentration of NH_2OH as those treated with CO but without caffeine. The excess respiration due to caffeine was inhibited to the extent of 31 per cent in the first hour and 38 per cent in the second hour. The CO excess in the matched muscles from the other leg was 86 per cent inhibited as shown in the table. The actual rates of respiration were higher in the case of the less inhibited respiration thus eliminating any explanation based on "saturation" phenomena as an explanation for the results. In another experiment 0.001 M NH_2OH caused only 39 per cent inhibition of the excess oxygen consumption of caffeinized muscle.

These facts are interpreted as an indication that although both respirations are sensitive to these reagents the CO oxidation is much more sensitive than oxidations presumably mediated by the cytochrome-oxidase system. Further discussion of this point appears below.

Discussion. The facts outlined above are taken as evidence that in frog muscle there is a dual effect of carbon monoxide: 1, inhibition of the Warburg-Keilin system, and 2, oxidation of the CO to CO_2. Whether or not the catalyst for CO oxidation is identical with any heretofore described intracellular oxidase awaits future study. It need hardly be emphasized that the normal function of this catalyst cannot be oxidation of carbon monoxide so that its identification may be considered of importance. A speculation entertained is a connection with the functioning of catalase. The activity of catalase is known to be inhibited by cyanide,

azide, and especially hydroxylamine (Keilin and Hartree, 1934; Blashko, 1935a). Furthermore the effects are obvious at much lower concentrations of inhibitor than those usually necessary for inhibition of cell respiration (cf. Blaschko, 1935b). The parallel with the experiments quoted above is obvious. The effect of CO itself on catalase is still unsettled since it seems to depend on the purity of the catalase preparation, and the presence or absence of oxygen or substances which inhibit the reoxidation of reduced catalase (Keilin and Hartree, 1938). But oxidation of the CO in the presence of catalase and another oxidase would not be entirely unexpected in view of the coupled oxidations studied by Keilin and Hartree (1935-36) which depend upon this type of coöperative action between catalase and an oxidase. However, experimental evidence is not yet available, and there is apparently no parallelism between the catalase content of tissues and their ability to burn CO. The most that can be said on the basis of the present experiments is that the oxidation of CO by muscle tissue is accomplished by a system separable from, but similar to, the normal respiratory systems. That it may indeed be closely related is indicated by the work with inhibitors and the demonstration by Negelein (1931) that CO can be burned to CO_2 by green and "mischfarbene" hemin in alkaline solutions at low O_2 tensions.

Since the resting respiration is unaffected by azide or hydroxylamine in concentrations which completely prevent the CO excess, the oxidation of CO as well as the extra respiration due to stimulation of the muscle (Stannard, 1939a, b) must be mediated by catalysts qualitatively different from the resting respiration as well as from each other. Cyanide inhibits both the resting respiration and the CO excess, it is true, but this is expected since the resting respiration is apparently a cyanide-labile, azide-stable respiration. Iodoacetamide (or IAA) does not inhibit CO oxidation in concentrations which have no effect on the resting respiration but does inhibit if this respiration is affected. However, this is not taken as evidence of a connection between the CO excess and the normal resting respiration partly because of the general destructive effect of these compounds in high concentration on all enzymes and partly because there is evidence that contradicts amply this interpretation.

Another line of evidence can be found by calculating the respiratory rate of the resting muscle in CO after subtraction of the CO used plus one-half mol of oxygen for oxidation of the CO. This can be done when the records include gas analyses of the oxygen and CO used. The average rate of respiration of the resting muscle in CO using all available data comes out to be 29 $mm^3/g./hr.$ which agrees well with the average of 30 $mm^3/g./hr.$ for normal resting muscle found in a long series of experiments. Of course the closeness of this agreement is an accident. However it seems justified

to propose that in resting muscle the oxidation of CO is superimposed quantitatively on the resting respiration. It is only when the muscle is "active" that the further complications of inhibition of the normal respiration are added.

There is some conflict between the line of reasoning taken here and the experimental results of Hursh (1936). Thus I have assumed that CO inhibits the Warburg-Keilin system at high CO tensions at least, and that the activity respiration of frog muscle traverses this system. Hursh did find less recovery oxygen in CO as the above would predict, but his lactate analyses showed no accumulation of lactate. Thus he concluded that the effect of CO was not essentially inhibitory. It may be possible to reconcile these data by finding that some other substance does accumulate after stimulation in CO or other evidence of incomplete recovery. Or the base line in CO may drop (i.e., less CO be burned) when the demand for oxygen is made by electrical stimulation of the muscle.

It appears from these experiments that CO is oxidized by muscle under a variety of conditions. It also appears that the oxidation is superimposed on partial inhibition of any increments in respiration when these are caused by the addition of dextrose, methylene blue, PPD, Nadi reagent, or caffeine. The inhibitory effect seems less pronounced when the increase in respiration is caused by lactate, pyocyanin, KCl, or o-chlorophenol indophenol. At least in these cases addition of CO causes a rise in respiration of nearly the same magnitude as that seen in resting muscle. A complete explanation of these phenomena requires future study. If it is true that CO inhibits the increments caused by certain substances and not at all those due to other substances it would be the first evidence that "stimulation" of muscle by these various means does not involve identical oxidative mechanisms. Such an interpretation requires ample experimental verification. It is possible, of course, that CO simply burns better in the latter instances so that the sum of inhibition and stimulation is higher. Obviously the point of this paper is the fact that CO is burned in both instances and inhibits the cytochrome oxidase when this is active.

It should be pointed out that the evidence shown in this and previous publications indicating "separable systems" of oxygen transfer indicates only their separate identity and not complete independence from one another. Functional interrelationships might well be expected in the complete biological system and may underlie many of the phenomena discussed above.

SUMMARY

1. The stimulation of frog muscle respiration by carbon monoxide has been confirmed and the analysis of the mechanism carried one step farther.

2. It is accepted that the stimulation is due to oxidation of the CO to CO_2, as shown by Fenn and Cobb, in spite of numerous attempts to disprove this somewhat unexpected phenomenon.

3. The CO excess is unaffected or not prevented by numerous substances which increase the normal respiration to high levels. Of these lactate, pyocyanin, KCl, and o-chlorophenol indophenol permit increases by CO above the prevailing rate of respiration, but dextrose, methylene blue, PPD, Nadi reagent, and caffeine do not.

4. In some cases at least the oxidation of CO is superimposed on inhibition of the cytochrome-cytochrome oxidase system. This was proven experimentally for the Nadi reagent by estimation of the indophenol blue formation, and for caffeine by direct analyses of the gases used. It is inferred that in dextrose and methylene blue as in caffeine the burning of CO is masked by an inhibition of the O_2 consumption by CO.

5. High CO tensions (95 per cent of an atmosphere) lead to no essential change in results when compared with 80 per cent CO-20 per cent oxygen mixtures.

6. IAA or iodoacetamide does not prevent CO oxidation except at concentrations high enough to inhibit the normal resting respiration.

7. Cyanide, azide, and hydroxylamine inhibit the CO excess, the latter two in concentrations that have no effect on the resting respiration and but a small effect on the activity respiration. The resting respiration is not affected by CO.

8. These experiments are interpreted as indicating that CO is oxidized in muscle by enzymes separable from the resting and the activity systems, but similar to the latter at least. Furthermore the effect of CO on muscle is dual, consisting, under certain conditions, of inhibition of the Warburg-Keilin system and superimposed CO burning.

9. A speculation as to the nature of the CO catalyst is included.

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THE EFFECT OF FAT AND CHOLESTEROL ON THE GROWTH OF YOUNG SALMON

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Preliminary experiments in this laboratory over a period of two years have shown that young chinook salmon cannot live on a dry diet adequate in every respect for the rat and with the addition of vitamin C unless it is supplemented with fresh meat or an extract of fresh liver. McCay and Dilley (1) reported that trout require a factor found in fresh meat in addition to the known vitamins which they called "factor H." The biochemistry of fish has been recently reviewed by McCay (2).

It was also found that the vitamin "B complex" as present in yeast is an essential requirement of young salmon. A high protein diet (70 to 80 per cent of the solids) produces a more rapid growth than a diet low in protein (30 per cent of the solids) and high in cooked starch, both being equal in salts and vitamins. Diets containing meat protein, extracted with boiling alcohol, gave more rapid growth with a lower mortality than purified casein or wheat gluten.

In feeding young salmon and trout in hatcheries considerable loss has been caused by a condition of "fatty" livers. In view of the work that has been published on the production of fatty livers in mammals by diets high in fat and cholesterol and the prevention of fat deposition by the feeding of pancreas or choline, it was considered of interest in the present paper to study the effects of these factors in feeding young chinook salmon.

Preparation of the diets. The present series of six experimental diets contain the basic components of meat protein, mineral salts, yeast, cod liver oil, and liver extract. Variable factors are starch, salmon oil, pancreatic extracts, cholesterol, and choline-hydrochloride.

The meat residue used as the source of protein in the present series of experiments was dried meat residue from the Valentine Meat Juice Company, Richmond, Virginia. It was extracted three times with boiling alcohol, washed with hot alcohol and then dried. The salt mixture used was Osborne and Mendel salt mixture with added copper sulphate.[1] The

[1] One hundred sixty-one thousandths gram of $CuSO_4 \cdot 5H_2O$ was added to the salt mixture for each 134.8 grams of calcium carbonate used.

liver extract used in all the diets as the source of "factor H" was prepared from ground fresh beef liver extracted with an approximate final concentration of 30 per cent alcohol.

The diets were prepared for feeding by thoroughly mixing all the solid material with the exception of the gelatin: Ten grams of gelatin were dissolved in water and diluted to a volume of 100 cc. at 35°C. after adding

TABLE 1

BASIC DIET	PARTS USED	VARIABLE FACTORS
Meat residue	55	Salmon oil
Salt mixture	4	Pancreatic extract
Sodium chloride	0.5	Cholesterol
Calcium carbonate	0.5	Choline-hydrochloride
Dried yeast	8	
Cod liver oil	2	
Liver extract*		
Gelatin	10	

DIET NUMBER	DIET	PARTS USED	DIET NUMBER	DIET	PARTS USED
9	Basic diet	80	12	Basic diet	80
	Starch (cooked)	20		Starch (cooked)	20
				Pancreatic extract†	
10	Basic diet	80	13	Basic diet	80
	Salmon oil	20		Salmon oil	20
				Cholesterol	2
				Pancreatic extract†	
11	Basic diet	80	14	Basic diet	80
	Salmon oil	20		Salmon oil	20
	Cholesterol	2		Cholesterol	2
1	Fresh raw liver‡	100		Choline-HCl	1

* Liver extract equivalent to 50 grams of liver added for each 100 grams of solids used in the preparation of the diets.

† Pancreatic extract equivalent to 50 grams of pancreas added for each 100 grams of solids used in the preparation of the diets.

‡ Control diet.

the tissue extract. The gelatin solution was then added to 90 grams of the mixed solids, mixed thoroughly and placed in the ice box to gel. This produced a food mixture of uniform texture and consistency that was easily fed with little waste. In feeding, the mixtures were grated through a wire sieve of appropriate mesh. The composite details of the diets are given in table 1.

FEEDING PROCEDURE. The fish used in each lot in this series of feeding tests were confined in separate troughs with uniform inflow of well aerated

water. The experiments were started with 1000 young chinook salmon (*Oncorhynchus tschawytscha*) in each trough, which number was reduced to 500 at the end of the twelfth week to avoid crowding. Each lot of fish was fed daily and given as much of the food mixture as they would con-

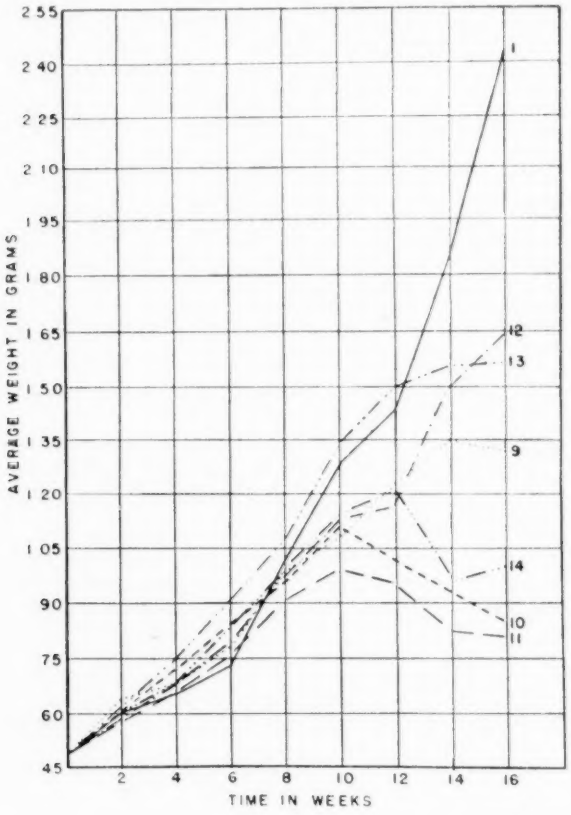

Fig. 1. The growth curves of young chinook salmon fed synthetic diets with and without added oil and cholesterol. The constituents of the diets are given in table 1.

sume without waste. The experiments were continued for sixteen weeks when they were discontinued because of the excessive mortality in some of the lots and a general slackening in the rate of growth. The results of the experiment as to growth are given in figure 1 and mortalities are summarized in table 2. In figure 1 the average weight of the live fish in each lot at the time of weighing is given.

OBSERVATIONS. The general growth of the fish in this series, as indicated by increase in average weight, was superior to the liver-fed lot during the first six weeks of the experimental period. From the tenth week to the end of the sixteenth week the rate of weight increase of the fish in each

TABLE 2

Cumulative mortality numbers by periods of two weeks each for fish fed experimentally

	LOT NUMBER						
	1	9	10	11	12	13	14
	Initial number of fish in each lot						
	2000	1000	1000	1000	1000	1000	1000
First period (12 weeks)							
February 9	16	14	19	17	29	8	36
February 23	37	22	25	25	37	17	46
March 9	51	24	34	38	45	19	52
March 23	61	24	35	40	49	20	56
April 6	72	26	38	40	53	26	81
April 20	75	32	63	56	55	27	96
Second period* (4 weeks)							
May 4	1	3	30	116	2	16	40
May 15	3	13	116	242	5	47	116
Total	78	45	179	298	60	74	212

* The number of fish in each lot was reduced on April 20 to avoid crowding. Lot 1 was reduced to 1000 fish and lots 9–14 were reduced to 500 fish each at the start of the second period.

TABLE 3

DIET NUMBER	PER CENT LIVER OF WET WEIGHT OF FISH	PER CENT OIL IN FISH BODY WITHOUT LIVER DRY WEIGHT	PER CENT OIL IN LIVER DRY WEIGHT
1	0.75	9.1	10.7
9	1.40	8.5	11.7
10	1.65	15.3	9.9
11	1.70	13.8	8.2
12	1.34	8.4	9.7
13	2.02	19.2	17.7
14	1.28	15.3	8.4

lot on the synthetic diets showed a tendency to diminish, falling below the average weight of those fish fed on a diet of liver and in some instances exhibiting a very marked decrease in average weight.

At the end of the experiment the fish were killed and the livers removed. Total fat was determined on the dried fish without the livers and on the livers separately. The results for fat determination are given in table 3.

McCay (2) investigated the digestion and utilization of fats by trout and concluded that trout could utilize considerable amounts of oil. They have "fed as much as 57 per cent of their diet in the form of fat without injury." Fats of low melting point, which are liquid at the body temperature of the fish, were digested better than fats of high melting point.

The effect of the addition of 20 per cent of salmon oil to the diet of young salmon can be seen by comparing the results on diets 9 and 10. The fish on diet 10 with added oil showed a much earlier break in the growth curve and higher mortality. With 2 per cent cholesterol in the diet group 11 had a still lower rate of growth and a much higher mortality. However, instead of an increase in liver lipids with increased fat and cholesterol in the diet there was a decrease in total liver lipids which might reflect a condition of general malnutrition of the fish.

Comparison of group 9 with group 12 and groups 10 and 11 with group 13 shows that an extract of pancreas improved the diet. The effect of pancreas was most marked in the case of diets containing both high oil and cholesterol or comparison of diets 11 and 13. That the effect of pancreas in improving the diet was not due entirely to choline may be seen by comparing the growth and mortality results of the groups of fish on diets 11 and 13 with the group on diet 14.

CONCLUSIONS

The growth of the fish fed the diets compounded from purified materials was inferior to that of the fish fed a diet of beef liver.

An alcoholic (30 per cent) extract of beef liver contains a substance other than the known vitamins which is essential for salmon. This is probably McCay's "H factor."

The addition of salmon oil to the basic diet produced inferior growth and excessive mortality.

The addition of alcoholic extract of beef pancreas increased the rate of growth and ultimate size obtained by the fish.

Cholesterol in the diet produced excessive mortality and poor growth.

Choline seemed to have a slight beneficial effect when added to a diet high in fat and cholesterol.

High fat (20 per cent) and cholesterol diets did not produce excessive fatty livers in young salmon under the conditions of the experiments given above.

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THE HISTAMINASE CONTENT OF THE TISSUES OF THE RAT AND THE EFFECT OF HISTAMINE PRETREATMENT

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In 1929 the discovery that animal tissue contained an enzyme which had the property of inactivating histamine was made by Best. An extensive study (Best and McHenry, 1930) revealed that the greatest activity is found in the kidney and in the mucosa of the small and large intestine in most species, and less activity in lung tissue. No activity was found in heart muscle, skin or stomach. Further studies by McHenry and Gavin (1932) showed that whereas the kidney of most species is rich in histaminase, the kidney of the rat and guinea pig contains none. Previous experiments (Rose and Browne, 1938) have shown that in the rat, destruction of intravenously injected histamine occurs mainly in the liver and kidney. The absence of histaminase in the kidney of this species as noted by McHenry and Gavin (1932) was confirmed at that time and the fact that the liver also was devoid of histaminase was noted. This suggested the possibility that some other tissue might be rich in histaminase or that another mechanism might be responsible for the inactivation of histamine in the rat.

There has been an accumulation of evidence to show that pretreatment of an animal with histamine will produce an increased tolerance not only to histamine (Eichler and Killian, 1931) (Karady, 1936) (Selye, 1937) but to many damaging procedures such as surgical shock (Rusnyak, Karady and Szabo, 1936) (Karady, 1938) and nephrectomy (MacKay and Clark, 1938). Smith (1939) was able to prevent anaphylactic shock in guinea pigs by histamine pretreatment, and recently Karady and Browne (1939) have been able to inhibit anaphylactic shock in guinea pigs by the use of histaminase. That pretreatment with histamine might produce increased tolerance by increasing the histaminase content of the tissues was suggested by MacKay and Clark (1938). Best and McHenry (1930) were however unable to alter the histaminase content of the kidney of the dog following pretreatment with histamine.

The studies about to be described were undertaken in an effort to determine the source of histaminase in the rat, and to note the effect of

[1] Aided by a grant from the Banting Research Foundation.

histamine pretreatment on the histaminase content of lung tissue in this animal.

METHODS. Rats of a "hooded" strain weighing between 140 and 180 grams were used. Under ether anesthesia and observing sterile precautions, the various tissues were removed and washed in saline. Stomach and small intestine were opened and thoroughly washed free of their contents. Excess fluid was removed by placing the tissues on large sheets of filter paper. The separate tissues were then finely ground up, and weighed amounts of this ground tissue were placed in Erlenmeyer flasks of 75 cc. capacity containing 20 cc. of phosphate buffer solution of pH 7.2. To this a definite volume of a histamine solution containing 1000 y (as base) per cubic centimeter was added and after the final addition of 10 drops of Toluol, the flask was incubated at 38°C. for 16 hours. After incubation, the flasks and their contents were heated to 90°C. for 3 to 4 minutes in a water bath, and then made up to 50 cc. by the addition of normal saline and assayed directly. Specimens of oxalated blood were incubated with histamine and buffer in flasks under the same conditions as described for tissues. After incubation, these specimens were extracted for histamine by the method of Barsoum and Gaddum (1935) as modified by Code (1937).

All specimens were assayed for their histamine content on the guinea pig ileum preparation suspended in Tyrode solution to which atropine sulphate was added in a concentration of 1×10^{-7}.

Since histaminase is inactivated by exposure to heat, certain specimens were first heated to 70°C. for five minutes before placing them in the incubator. As a further control, flasks containing histamine and buffer without the addition of tissue were incubated in order to exclude the possibility of spontaneous inactivation of histamine occurring under these conditions.

It should be noted that although the weight of tissue and original quantity of histamine incubated may vary, in most cases the same weight of tissue, namely, 0.5 gram and the same amount of histamine, that is 1000 y have been used in order to clarify results, and that in all the tables, the values for histamine indicate the amount destroyed in 16 hours and are expressed as histamine base unless otherwise indicated.

EXPERIMENTAL RESULTS. A. *Histaminase powder.* In preliminary experiments, use was made of a standard commercial histaminase preparation (Torantil)[2] supplied in ampoules containing approximately 15 mgm. which is capable of destroying 1 mgm. of histamine HCl in 24 hours. Two experiments were carried out. In the first the contents of one ampoule were incubated with increasing amounts of histamine. In the second the

[2] Supplied through the courtesy of the Winthrop Chemical Company and Dr. H. Cave, Montreal.

amount of histamine was kept constant but the quantity of histaminase was varied. The results are shown in table 1. It will be observed that the destruction of histamine takes place in a direct ratio to the amount of histaminase present and that further, the destruction of histamine by a fixed amount of histaminase increases in direct ratio to the concentration of histamine present.

B. *Ability of various tissues to inactivate histamine.* In order to determine which tissues were capable of destroying histamine, five experiments were carried out in each of which a group of five adult male rats was used. The tissues studied were lung, liver, kidney, spleen, small intestine, stomach and blood. Of these, histamine was destroyed only by lung and small intestine. There was no destruction of histamine in those flasks containing specimens of lung or small intestine which were heated to 70°C. prior to incubation, or in the flasks containing histamine alone (see table 3).

TABLE 1

Potency of torantil (T. 360) a histaminase preparation

AMOUNT OF POWDER	HISTAMINE INCUBATED	HISTAMINE DESTROYED
mgm.	*γ*	*γ*
(one ampoule)		
±15	1000	440
±15	2000	720
±15	3000	1000
±15	4000	1200
±15	5000	2600
10	1000	300
15	1000	520
20	1000	650
30	1000	890

TABLE 2

Histamine content of solutions after incubation with lung tissue as obtained by direct assay and following extraction

WEIGHT OF TISSUE	HISTAMINE DESTROYED BY DIRECT ASSAY	HISTAMINE DESTROYED AFTER EXTRACTION
gram	*γ*	*γ*
0.5	700	
0.5	650	
0.5	750	
0.5		700
0.5		680
0.5		720

Original amount of histamine 1000 γ.

Since lung tissue is much easier to work with and in this species contains more histaminase than the intestine, it was decided to use this tissue for the remainder of the experiments.

C. *Reliability of the method.* Feldberg and Kellaway (1937) showed that simple extracts of ground up lung tissue could be assayed directly for their histamine content without the necessity for chemical extraction. In order to obviate the possibility that the histamine was simply adsorbed to lung tissue and not really destroyed the following experiment was performed. Six specimens of the same lung tissue each of 0.5 gram in weight

were incubated with histamine as described above. After incubation, three of these specimens were made up to volume in the usual way. The remaining three were extracted for histamine by the method of Best and McHenry (1930). All six were then assayed and as will be seen in table 2 there was no essential difference in the results, indicating that chemical extraction is not essential.

As a further indication that destruction of histamine was due to the presence of lung tissue, varying amounts of the same sample of tissue were incubated with a fixed quantity of histamine. It will be noted in table 4 that as the quantity of tissue was increased, a greater amount of histamine was destroyed. It will be noted that although the amount of histamine destroyed by a given weight of tissue varies with different

TABLE 3

Ability of various tissues of the rat to inactivate histamine

LUNG	HISTAMINE DESTROYED	INTESTINE	HISTAMINE DESTROYED	LIVER	HISTAMINE DESTROYED	KIDNEY	HISTAMINE DESTROYED	SPLEEN	HISTAMINE DESTROYED	BLOOD	HISTAMINE DESTROYED
grams	*γ*	*grams*	*γ*	*grams*	*γ*	*grams*	*γ*	*grams*	*γ*	*cc.*	*γ*
1.5	500	6.5	400	2	0	1.5	0	1.0	0	5	0
1.3	450	5.8	425	3	0	2.0	0	0.5	0	7	0
1.0	400	7.6	700	5	0	1.0	0	1.5	0	10	0
1.5	470	4.0	350	5	0	1.5	0	2.0	0	10	0
0.5	350			4	0	4.0	0	1.7	0	10	0

Original amount of histamine 800 γ.

experiments, the amount of variation is not great and on the average 0.5 gram of tissue will destroy 730 γ of histamine in 16 hours.

D. *Effect of histamine pretreatment.* Four groups of five rats each were pretreated with histamine for a period of 10 days. The first two received an amount known to be effective in suppressing the water retention occurring in rats following an intravenous dose of histamine (Howlett and Browne, 1937). They were given 20 mgm. of histamine HCl on the 1st day followed by 40 mgm. daily for nine successive days. The second two groups received the following dosage: 20 mgm. on the first day followed by an increase on each successive day, i.e., 30, 40, 60, 80, 80, 80, 80, 80 and 80 on the tenth day. All injections were given subcutaneously. Six hours after the last injection, the animals were killed and the tissues removed for incubation.

Various other tissues besides lung were removed at the same time in order to preclude the possibility that pretreatment might cause histaminase formation in other tissues. As in the first experiments, there was no

destruction of histamine by any of the tissues with the exception of lung and intestine. A comparison of the results obtained with tissue from pretreated animals shows that there is little variation as compared to that in normal tissue (see table 5).

TABLE 4

Histaminase activity of lung tissue from intact rats

LUNG TISSUE	HISTAMINE DESTROYED
grams	*γ*
0.05	200
0.1	300
0.6	825
1.0	875
Control	0
0.05	200
0.1	300
0.5	700
1.0	875
Control	0
0.1	200
0.1	200
0.5	700
1.0	875
1.0x	0
Control	0
0.05x	0
0.1	200
0.5	400
Control	0
0.5	800
0.5	800
0.5	800
0.5x	0
Control	0

Specimens marked x were heated to 70°C. before incubation.

Original amount of histamine 1000 γ.

TABLE 5

Effect of histamine pretreatment on the histaminase content of the lung of the rat

WEIGHT OF TISSUE	HISTAMINE DESTROYED	DATA
grams	*γ*	
0.5	650	Normal animals
0.5	600	
0.5	700	
0.5	600	
0.5	600	Pretreated animals
0.5	600	
0.5	600	
0.5	600	
1.0	800	Normal animals
1.0	875	
1.0	800	Pretreated animals
1.0	875	
0.5	650	Normal animals
1.0	800	
0.5	650	Pretreated animals
1.0	850	

Original amount of histamine 1000 γ.

DISCUSSION. Although an enzyme system specific for histamine in-

activation is present in certain tissues of the rat, namely, lung and small intestine, it does not seem probable that the rapid inactivation of histamine occurring in the rat following an intravenous injection of the amine can be accounted for by this enzyme. In support of this is the fact that rat kidney and liver, both of which contain no histaminase, are responsible for the greater part of the destruction of intravenously injected histamine.

One of us (Rose) has shown that the rate of disappearance of intravenously injected histamine is not altered in the histamine pretreated rat as compared with the nontreated animal. This, and the fact that histamine pretreatment does not alter the histaminase content of lung tissue indicates that increased resistance to histamine and conditions such as surgical or anaphylactic shock following pretreatment with histamine must be accounted for by some mechanism other than an increase of histaminase.

It has been shown that histamine may be destroyed by other mechanisms (Holtz, 1937; Garan, 1938). Certain changes in the response of the organism following pretreatment may be accounted for on a basis of altered response of smooth muscle. This is illustrated by the fact that the usual drop in blood pressure in the cat following an intravenous injection of histamine is changed to a rise following histamine pretreatment (Karady, 1936).

Recently it has been shown that pretreatment with histamine in the rabbit will prevent the decrease in the blood volume usually observed after either histamine or surgical shock (Karady, 1938). It has also been noted that the water retention which may be produced in rats following the intravenous injection of histamine may be greatly diminished if the animals are first pretreated with histamine (Howlett and Browne, 1937).

The suggestion that diminished resistance in the rat following nephrectomy is due to the fact that this organ is rich in histaminase, and that pretreatment with histamine may possibly increase the histaminase content as suggested by MacKay and Clark (1938) is untenable in the light of the above findings.

SUMMARY

A method for the determination of histaminase in rat tissue is given. Of all the tissues studied, only lung and intestine are capable of destroying histamine. No increase in the histaminase content of lung tissue is observed, following pretreatment of the rat with histamine.

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